Volume 13, Issue 4, December 2023
pISSN 2158-0510 eISSN 2158-0529

IJBM

International Journal of BIOMEDICINE

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International Journal of Biomedicine (ISSN 2158-0510) is published four times a year by International Medical Research and Development Corp. (IMRDC), 6308, 12 Avenue, Brooklyn, NY 11219 USA

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International Journal of Biomedicine 13(4) (2023) 197-206
http://dx.doi.org/10.21103/Article13(4)_RA1

REVIEW ARTICLE

INTERNATIONAL JOURNAL OF BIOMEDICINE

Kisspeptin Hormone: Revolution in Reproductive System Physiology

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Abstract

The control of reproduction has been attributed to the actions and feedforward of the sex steroids gonadotropin-releasing hormone (GnRH), luteinizing hormone (LH), and follicle-stimulating hormone (FSH). However, recent findings, including the identification of kisspeptin neurons and a kisspeptin-GnRH-LH/FSH axis, have prompted a reevaluation of reproductive regulation. At first, the *KISS1* gene encoding kisspeptin was thought to belong to a group of genes called metastasis suppressors. Vertebrate and mammalian genomes have been enriched with *Kiss* and *KissR* gene variations during the last two decades. In 2003, kisspeptins and their receptor, *KISS1R*, and their role in the neuroendocrine-reproductive axis were discovered. This finding radically altered our understanding of reproductive physiology. These discoveries support the role of kisspeptins and their receptor as gatekeepers of sexual maturity at the outset of puberty and as key processors in the adult-life dynamic control of the gonadotropic axis. The significance of kisspeptin signaling in spermatogenesis and sperm quality is still debatable, even though *Kiss1* and *Kiss1R* are expressed peripherally in the testes. Numerous processes, including steroidogenesis, follicular maturation, ovulation, and ovarian senescence, are affected by kisspeptin activity. Therefore, kisspeptin analogs (both agonists and antagonists) may be useful as therapies for those with disorders affecting the reproductive system. This overview focuses on the evolution, localization, and reproductive importance of the Kiss–KissR pair. **(International Journal of Biomedicine. 2023;13(4):197-206.)**

Keywords: kisspeptin • KISS1• KISS1R• kisspeptin analogs • kisspeptin-GnRH-LH/FSH axis

For citation: Abdelaziz MA. Kisspeptin Hormone: Revolution in Reproductive System Physiology. International Journal of Biomedicine. 2023;13(4):197-206. doi:10.21103/Article13(4)_RA1

Abbreviations

GnRH, gonadotropin-releasing hormone; **LH**, luteinizing hormone; **FSH**, follicle-stimulating hormone; **HPG** axis, hypothalamus-pituitary-gonadal axis; **HH**, hypogonadotropic hypogonadism; **KsRE**, kisspeptin-response element; **KNDy**, kisspeptin/neurokinin B/dynorphin; **SPZ**, spermatozoa; **IVF**, in vitro fertilization; **OHSS**, ovarian hyperstimulation syndrome.

Introduction

The *KiSS-1* gene (HGMW-approved symbol, *KISS1*) encoding for a hydrophobic 145 amino acid protein called kisspeptin was discovered in 1996 by Lee et al.[1] as a malignant melanoma metastasis-suppressor gene. It was named "*KISS1*" so that everyone would know where it was discovered, namely in Hershey, Pennsylvania, home of the famous Hershey Kisses chocolates. The "SS" in KISS1 stands for "suppressor sequence" to honor its role in gene regulation. In humans, the *KISS1* gene is located on the long (q) arm of chromosome 1 at q32 with four exons.[1,2] Post-translational processing of the 145-amino-acid intermediate prepropeptide encoded by *KISS1* yields four physiologically active peptides with distinct molecular weights: kisspeptin-54, kisspeptin-13, kisspeptin-10, and kisspeptin-14 (Figure 1).[3] All these peptides have a C-terminal region that contains an Arg–Phe–NH2 motif characteristic of the RF-amide peptide family, which allows them to bind to and fully activate the kisspeptin receptor (KISS1R).[4-6] In many mammals, kisspeptins stimulate the release of GnRH (and subsequently the secretion of FSH and LH) by binding to the KISS1R, which belongs to the G-protein-coupled receptor family.[7]

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This seven-transmembrane receptor is structurally similar to the transmembrane region of galanin receptors, with ~40% sequence identity.[4]

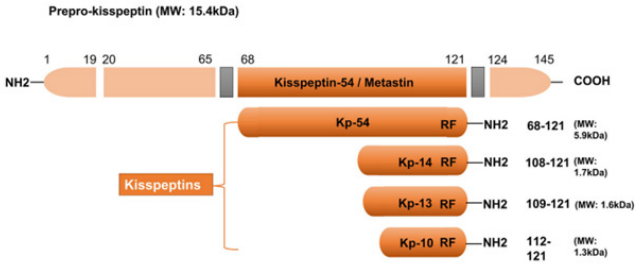

Fig. 1. *Human kisspeptins, products of the KISS1 gene. "Different kisspeptins are generated by the cleavage from a common precursor, the prepro-kisspeptin. The prepro-kisspeptin contains 145 amino acids, with a 19-amino acid signal peptide and a central 54-amino acid region, kisspeptin-54 (Kp-54; formerly termed as metastin). Further cleavage of metastin generates kisspeptins of lower molecular weight: kisspeptin-14 (Kp-14), Kp-13, and Kp-10. All kisspeptins contain the RF-amide motif that is able to bind and activate kisspeptin receptor."*[3]

Kisspeptins were utilized universally to describe this class because of their structural similarity and shared ancestry as KISS1-derived peptides.[8,9] Kisspeptin-54 was initially termed "metastin" because of its capacity to inhibit tumor metastasis. This peptide has been considered as the major product of the human KISS1 gene.[10] The larger peptide comprises some variability among species, whereas the 10 amino acid C-terminus peptide is well conserved and binds to and activates KISS1R.[1,5,6,11]

A later cloning effort resulted in identifying the human homolog of KISS1 as a putative receptor for KISS1-derived peptides in several databases. Multiple laboratories have independently identified and/or investigated the physiological functions of KISS1R (also known as GPR54, AXOR12, hOT7T175, CPPB1, and HH83).[12] A revolution in reproductive physiology began with the 2003 discovery of kisspeptins and their receptor in the neuroendocrine-reproductive axis.[13,14] These findings support the role of kisspeptins and their receptor as gatekeepers of sexual maturity at the outset of puberty and as key processors in the adult-life dynamic control of the gonadotropic axis.

De Roux et al.[15] reported on a family affected by HH in 2003. These individuals shared a deletion of 155 nucleotides in the orphan receptor gene *GPR54* [or *KISS1R*], encoding a G protein-coupled receptor, which impeded the onset of puberty and the development of reproductive organs. Using genetic engineering, Seminara et al.[16] induced the same defect in the *Gpr54* gene of mice, discovering disruptions in puberty and reproductive processes. This demonstrated the critical role of *GPR54* in reproductive regulation. Kisspeptin-54 and kisspeptin-10 were found to be *GPR54* ligands.[2,17,18] It was subsequently shown that hypothalamic neurons carrying kisspeptin send axons to GnRH neurons that express KISS1R.[19-22] In addition, estrogen receptor alpha (ERα) expression was detected in kisspeptin neurons.[23] These results showed that kisspeptin neurons are downstream of GnRH neurons and receive direct peripheral feedback of sex steroid hormones via KISS1R. Multiple neuropeptides, such as GABA and glutamate (which is also input to GnRH), have been proposed to be involved with kisspeptin neurons; however, these are better understood as neurons involved with adjusting the function of the large performing kisspeptin neural cells than as neurons downstream of kisspeptin neurons. Because of this, kisspeptin is an integral part of the feedback system that regulates the GnRH-LH/FSH axis.

In the last 25 years, researchers have made significant progress in understanding the signaling processes of biological systems thanks largely to kisspeptins and their receptor, Kiss1R.[24] In 2003, it was discovered that HH, characterized by deficiencies in GnRH secretion, gonadotropin discharge, and infertility, is caused by inactivating mutations in the human *KISS1R* gene.[14,25] Its biological role has broadened from cancer progression to reproduction, fertility, and energy homeostasis since the system was first characterized in non-mammalian vertebrates,[26,27] and Kiss1 and Kiss1R were found to be expressed (at the mRNA and protein levels) in peripheral tissues such as gonads, placenta, pancreas, adipose tissues, liver, and vasa.

Kiss1 and Kiss1R in Regulating Fertility

The generation of gametes is regulated by endocrine, paracrine, and autocrine communications between the pituitary (which secretes gonadotropins like FSH and LH, among others) and the gonads (which secrete sex steroids like testosterone). Humans and animal models with mutations in the *KISS1/KISS1R* genes exhibit precocious puberty and HH due to stimulation or dysfunction of the hypothalamic HPG axis.[14,25,28-30] Specifically, the neurohormone GnRH is produced and secreted by neuronal populations regulated by kisspeptin in the arcuate nucleus and the anteroventral periventricular nucleus.[31]

Kisspeptin's influence on *GnRH* gene expression is mediated by dynamic chromatin changes that it creates.[32,33] Kisspeptin-response element (KsRE) in the mouse genome, located at positions -3446 and -2806, and on GnRH-neuronal cell lines, and mutant mice were used to locate an enhancer of the *GnRH* gene. Orthodenticle Homeobox 2 (Otx-2) is a transcription factor whose binding site is encoded by the *KsRE* gene. To activate *GnRH* gene transcription, kisspeptin treatment first creates nucleosome-depleted DNA in KsRE, which then promotes Otx-2 gene transcription and protein synthesis.[32] Kisspeptin influences recognized indicators of active chromatin, but it has no impact on repressive markers of genes; the fact that it enhanced acetylation of histone 3 (H3) at lysine (K)14 and (K)27 and trimethylation of H3K4 inside the KsRE is proof of this; however, it had no impact on the dimethylation of H3K9 in the KsRE. Kisspeptin was shown to have a positive effect on transcriptional activation of the *GnRH* gene by facilitating the functional establishment of a chromatin loop between the KsRE and the downstream-located neuron-specific element; this was discovered by chromosome conformation capture analysis.[33]

In addition to influencing reproduction through epigenetic mechanisms,[34,35] kisspeptin neurons relay several environmental stimuli down the HPG axis and serve as an

intermediary in the sex-steroid-driven feedback mechanisms.[36] Toxins in the diet and the environment may have a significant impact on fertility by modulating the function of neurons that produce Kiss. The finest illustration of how epigenetics may be utilized to regulate a biological process such as puberty is provided by the NAD+-dependent deacetylase Sirtuin 1 (SIRT1), which genetically modifies Kiss1 expression in the hypothalamus.[37] SIRT1 works as a metabolic sensor that activates or represses gene expression via pathways of energy availability, molecules involved in transcription, and the deacetylation of histone proteins, all of which are essential for successful reproduction.[38]

The Onset of Puberty and GnRH Regulator

Among its many functions, kisspeptin is well recognized for guarding the entrance to puberty and regulating the release of pulsatile GnRH. The HPG axis is controlled by the GnRH neurons that are located in the basal forebrain. Axon terminals in the median eminence of the hypothalamus release pulsatile GnRH into the circulation of the hypophysis, which in turn triggers the pulsatile synthesis of LH and FSH.[39,40] These hormones aid in the growth of the testes and ovaries, allowing for the production of sperm and eggs. Even though negative or positive feedback from gonadal sex hormones on GnRH release is well documented, GnRH neurons lack estrogen receptors.[40] Since kisspeptin neurons in the anterior ventricular preoptic region (AVPP) are known to regulate GnRH neurons upstream, the observation that these neurons express estrogen receptor (ER2) suggests that estrogen regulates GnRH indirectly via kisspeptin.[41] Research on the role of kisspeptin in reproduction in animals has exploded thanks to the discovery of Kiss1's modulation of GnRH via sex steroids. Kiss1 mRNA is reported to grow significantly in the AVPV neurons of both male and female mice from juvenile to adulthood. Adult mouse GnRH neurons are stimulated by kisspeptin and become sensitive throughout postnatal development, although co-expression of the *Kiss1R* does not alter from juvenile to adulthood.[42] If the kisspeptin receptor is blocked or deleted during the juvenile period, puberty development is thrown off. The pulsatile GnRH and LH surge is entirely suppressed in male and female mice lacking *Kiss1*.[43]This demonstrates that Kiss-KissR's important function in generating GnRH pulses is crucial for puberty in mammals. It is known that the reproductive axis is driven by neurokinin-B, dynorphin A, and melanocortin, as well as by genetics, the environment, and peripheral cues; however, neither the cause nor the time of pubertal transition is well understood.[44,45] Numerous studies in animals, especially mice, demonstrate that kisspeptin is a potent secretagogue of hypothalamic GnRH, which governs the onset of puberty.[46]

Kisspeptin and Its Physiology

The reproductive system is controlled by a web of complex feedback loops between the hypothalamus and pituitary gland. In females, the ovaries play a pivotal role in controlling hormone levels through the hypothalamic-pituitary-ovarian axis. The pituitary gland secretes FSH and LH in response to pulsed hypothalamic release of GnRH, a mechanism known as the hypothalamic-pituitary axis.[47] Together, these gonadotropins have a synergistic effect on steroidogenesis in the ovaries. Pulsatile GnRH secretion is the primary regulator of reproductive processes. Regulation of GnRH occurs through both direct and indirect mechanisms. Neuropeptides and neurotransmitters, such as galanin, neuropeptide Y, neurokinin B (NKB), nesfatin-1, kisspeptin, corticotropin-releasing hormone, and norepinephrine, all have roles in modulating GnRH function, and many more.[48] Particularly, kisspeptin seems to play an important role in controlling fertility.

Several regions of the human brain, including the hippocampus, anterior pituitary gland, and hypothalamus (especially the infundibular nucleus), exhibit significant levels of KISS1R. Additionally, the pancreas, liver, ovaries, and adipose tissue all have high levels of KISS1R expression in addition to the brain.[9] Kisspeptin secretion and the complex mechanisms by which it acts are currently the subject of intensive study.

KNDy Neurons and Their Physiology in the Regulation of the Hypothalamic Reproductive System

The reproductive axis (hypothalamus-pituitary-ovarian axis) greatly depends on GnRH pulsatile generation. Most human neurons that produce GnRH are located in the hypothalamic infundibular nucleus; from the infundibular nucleus, these neurons travel to the median eminence.[49]

The pulsatile release of gonadotropins (FSH and LH) is stimulated by GnRH secretion from this anatomical location. Considerable progress has been made in studying the effect of kisspeptin on GnRH production since its discovery and identification in the hypothalamus. In the infundibular nucleus, KNDy neurons co-express the opioid peptides dynorphin (DYN), neurokinin B (NKB), and kisspeptin. The first known clusters of KNDy neurons were discovered in a sheep's brain in 2007.[50] Subsequent research in the human hypothalamus found anatomically similar clusters of neurons. Research has shown that KNDy neurons are critical for controlling GnRH neuron activity.[51] It has recently been discovered that NKB and DYN neurons regulate kisspeptin's effect on GnRH secretion.[52] Specifically, NKB is essential for starting kisspeptin pulses, which triggers GnRH secretion in the body. The release of kisspeptin is inhibited by DYN neurons, which reduces GnRH's pulsatility (Figure 2). KNDy neuron expression has been shown to be sex-specific. The researchers demonstrated the presence of KNDy cell populations and sexual dimorphism using immunohistochemistry and deep brain imaging; female participants had higher KNDy in the arcuate nucleus (ARC) than male specimens.[53] Not only do humans exhibit sexual dimorphism, but so do other primates and rodents. Understanding how ovarian sex steroids affect KNDy neuron function is critical for appreciating their larger purpose. KNDY neurons express a wide variety of receptors for steroid hormones, including NKB, DYN, estradiol receptors, and progesterone receptors. Because of this, the KNDy neuron can serve as the reproductive system's master integrator of systemic feedback.[54]

Thus, ovarian steroids can regulate *KISS1* expression in the hypothalamus. In turn, kisspeptin triggers the pulsatile release of GnRH. Ovarian steroid secretion during the early follicular phase can inhibit GnRH release; on the other hand, LH pulsatility might be triggered by increased estradiol

production during the late follicular phase, increasing GnRH release.[55,56]

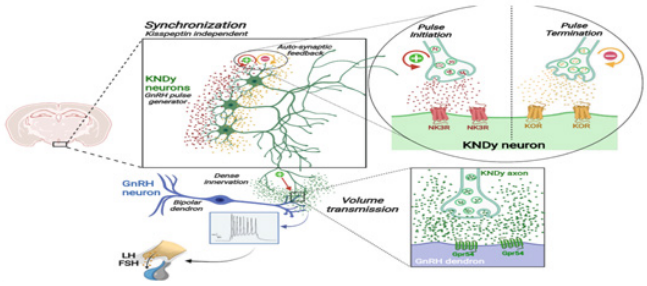

Fig. 2. *"KNDy neurons and GnRH pulse generation: a schematic overview of the fundamental processes. KNDy neurons, with their many collateral connections, create a highly interconnected network that coordinates the release of kisspeptin from GnRH neurons in order to generate GnRH pulses. When it comes to pulse formation, NKB is essential for starting kisspeptin pulses, whereas Dyn has the opposite effect and is in charge of stopping them. While entire GnRH pulses may be generated without kisspeptin input to GnRH cells, synchronization of KNDy neurons does need it. Transmission between KNDy neurons and GnRH dendrons near the edge of the median eminence is expected to take place through volume transmission, as shown by recent data."*[14]

Expression of Kiss1 mRNA in the rat ovary varies depending on the ovulatory increase in gonadal hormones,[56] peaking during the pre-ovulatory phase. Furthermore, when prostaglandin synthesis was inhibited, ovulatory dosages of human chorionic gonadotropin could not stimulate *Kiss1* expression in the rat ovary, which is known to greatly impair ovulation.[57] These results hinted, if indirectly, to a possible function for local kisspeptins in regulating ovulation. This theory is supported by the observation that anti-kisspeptin infused intra-ovarian decreased the quantity of corpus luteum, a marker of ovulation, but kisspeptin injected directly into the ovary had the opposite effect.[58] Kisspeptins have been found in clinical studies to promote egg maturation and ovulation in animals,[59] reduce the likelihood of developing ovarian hyper-stimulation syndrome, and promote oocyte maturation in high-risk individuals.[60] Parenteral injection of kisspeptin also produces substantial gonadotropin responses, making it challenging to disentangle the local and central mechanisms responsible for these results.

Taken together, these findings and evidence of Gpr54 expression in oocytes in rodent, canine, and porcine species reveal a potential role for kisspeptin's direct effects in regulating ovulation, a lack of which would lead to premature ovulatory failure, which is analogous to premature ovarian insufficiency (POI). However, there is insufficient evidence of whether kisspeptins act on oocytes directly or indirectly. Selective oocyte depletion of Gpr54 causes the progressive POI-like syndrome, manifesting as increased atresia of big antral follicles and early anovulation, but no effect on resting follicle numbers or development. [61]

Kiss1 and Kiss1R in Testis and Spermatozoa

Multiple types of signaling—autocrine, paracrine, and endocrine—contribute to the complex process of spermatogenesis. In the interstitium, Leydig cells create sex steroids, and growing germ cells get structure and nutrition from Sertoli cells.[62] This relies heavily on the coordinated proliferation and death of germ cells as well as the meiotic division and differentiation processes in which these cells play a central role. In mammalian and non-mammalian vertebrate testes, the kisspeptin system's location inside the testis, possible autocrine and paracrine activities, steroid synthesis, the development of sperm production, and sperm functions have all been studied.[63-65]

Blood levels of kisspeptin in males vary depending on their fertility, with levels being much greater in fertile males than in infertile ones.[66] In particular, people with HH have elevated plasma kisspeptin levels, but these levels decrease following GnRH replacement treatment because the hypothalamic sex-steroid feedback mechanisms have been restored.[67] Testicular Kiss1R signaling is essential for steroidogenesis; however, in patients with *KISS1R* inactivating mutation,[68,69] gonadotropin stimulation does not necessarily restore testosterone synthesis and spermatogenesis, similar to how *Kiss1R*-/- knockout animals still need an intra-testicular kisspeptin signal for spermatogenesis.[70] Spermatogenesis cannot be returned by activating the GnRH-secreting neuron and reactivating the Kiss1R gene. Male *Kiss*-/- mice with HH treated with testosterone had normal levels of plasma and intra-testicular testosterone and sustained spermatogenesis until sperm were produced that could fertilize eggs in vitro. Still, these animals were unable to inseminate females.[71] Kisspeptin can stimulate spermatogenesis in most intact animal models after being administered.[72-74] However, persistent overstimulation of the kisspeptin-dependent HPG axis leads to testis damage[75] and shuts off the HPG axis via desensitization of the Kiss1R in the testes.[76] It has been hypothesized that luteinizing hormone (LH)-dependent intra-testicular kisspeptin synthesis has a synergistic effect on postnatal testicular development and Leydig cell maturation in rats.[77] Kisspeptin is produced centrally and activates the HPG axis, which causes the hypothalamus to secrete GnRH and the anterior pituitary to produce gonadotropin LH. Kiss1 expression is upregulated by LH signaling in Leydig cells through cyclic adenosine monophosphate and protein kinase activation A pathway.[78,79] Effects on GnRH expression in Leydig cells in vitro have been documented, and the intra-testicular GnRH system, estradiol, and testosterone levels of non-mammalian vertebrates have been shown to be modulated.[73,80] Reports suggesting that kisspeptin affects the progression of spermatogenesis have been corroborated by recent investigations utilizing ex vivo testes explants and more physiological conditions, such as co-culturing germ and somatic cells.[72,73,80,81]

However, the importance of the intra-testicular kisspeptin system remains unclear and up for debate, although in vivo, ex vivo, and in vitro data suggest a possible role in Leydig cell function, steroid secretory activity, or spermatogenesis.[64] Goat, hamster, mouse, and rat epididymis have been defined, and the system is expressed in humans, bulls, rodents, and frogs.[65,82] However, the importance of kisspeptin in achieving spermatozoa (SPZ) competence for fertilization has not been thoroughly explored. Kiss1R is predominantly found in the post-equatorial area of the human brain[83] and the acrosomal region of the mouse brain.[84]

The kisspeptin system in SPZ can be controlled by specific agonists/antagonists under physiological conditions; however, this has only been described in human and mouse SPZ.[84] Human sperm hyper-motility was reported to be affected by kisspeptin-13.[83] However, kisspeptin-13 was found to affect the fertilization potential of rat SPZ obtained from the cauda epididymis.[84] Previous research studied the kisspeptin system in dog and rat SPZ,[82,85]) providing evidence of Kiss1R trafficking in SPZ head during the transit from caput to cauda epididymis.

Using a canine model that is somewhat close to humans, and using a combination of flow cytometry, epifluorescence microscopy, and Western blot on specific membrane protein fractions, scientists were able to identify Kiss1R on membrane-intact SPZ extracted from the epididymal tail. For instance, the presence of Kiss1R on the surface of SPZs coincides with the growth of protamination rate and motility, two characteristics of fully developed epidydimal cells.[85] From the caput's posterior SPZ head region, Kiss1R travels to the tail's perforatorium, as shown by an analysis of permeabilized SPZ from the caput and tail epididymis of rats.

Kiss1 was detected in canine and rat epididymal fluid using a dot blot assay.[82] High levels of Kiss1 were detected in the epididymal fluid and plasma of rats using a more sensitive ELISA technique, which served as a positive control.[82] Kiss1R trafficking is considered to be a hallmark of proper sperm production, and kisspeptin signaling may be a signal for SPZ storage in the epididymis. The lack of functional data on the activity of Kiss1R in SPZ acquired from different epididymal tracts in both healthy and pathological situations is a significant drawback of the previous research and requires more study. Kisspeptin levels in the seminal plasma were positively associated with sperm quality in a large sample of healthy males, according to the study's authors. The concentration of kisspeptin in seminal plasma was much greater than that in blood plasma. The occurrence of SPZ problems in animal models suggests that measuring kisspeptin levels in the seminal plasma of normal-spermic, sub-fertile, and infertile males and SPZ-deficient animals may be interesting.

Kisspeptin Analogs

Kisspeptin Agonists

There has been a rise in the use of kisspeptin analogs to treat endocrine diseases. Kisspeptin agonists are most useful in assisted reproductive technologies for inducing ovulation. Couples struggling with infertility may be hesitant to try IVF because of the risk of serious complications, such as OHSS.[86] The complications of OHSS include kidney failure, acute respiratory distress syndrome, swollen ovaries, and even death.[87] Medication used to promote ovulation for oocyte extraction in IVF regimens is the primary cause of OHSS.[88,89]

In this aspect, kisspeptin may be a more secure option than current IVF protocols. Ovulation necessitates kisspeptin due to its role in stimulating the preovulatory LH surge. In women undergoing IVF, kisspeptin analogs (such as kisspeptin-54) have been shown to be effective in stimulating oocyte maturation. The delivery of kisspeptin-54 resulted in egg maturation in a study by Jayasena et al.[90] The average number of developed eggs per patient is also associated positively with the amount of analog given to each individual.[90] The conventional medicines used to stimulate oocyte maturation were compared to kisspeptin-54 in a head-to-head trial conducted by Owens et al.[91] Serum levels of FSH and LH measured after the kisspeptin administration were more suggestive of a normal hormonal cycle than those measured after administering conventional medications. Abbara et al.[60] found that while the LH surge following the kisspeptin administration is smaller than that of a standard GnRH agonist, it may increase oocyte maturation by acting on kisspeptin receptors in the ovaries. Abbara et al.[92] also gave kisspeptin-54 to 60 women at high risk of OHSS to test the hormone's capacity to stimulate oocyte maturation in preparation for in vitro fertilization. Ninety-five percent of treated women matured their oocytes, and no women had mild, moderate, or severe OHSS. In women who are at high risk of OHSS, this finding supports the use of kisspeptin-54 to stimulate oocyte maturation during IVF. Beyond *their* use to stimulate ovulation, kisspeptin agonists provide promise in the treatment of people with disorders linked with decreased LH output. Plasma levels of luteinizing hormone have been studied by Whitlock et al.,[93] who investigated the effects of kisspeptin and kisspeptin receptor agonists. Serum LH levels in sheep increased significantly after treatment of both. Kisspeptin agonists may be useful in treating illnesses such as hypothalamic amenorrhea; according to these findings, women who suffer from functional hypothalamic amenorrhea may benefit from receiving an intravenous infusion of kisspeptin-54 since it has been shown to boost LH pulsatility. This paves the way for additional research into the efficacy of kisspeptin-based treatments in treating functional hypothalamic amenorrhea in females.[94] Kisspeptin was also studied in relation to pubescence; prepubertal bull calves were given analogs of kisspeptin, as well as acute and subacute doses of kisspeptin, and both methods of administering kisspeptin analogs were linked to increases in LH levels. However, subacute treatment of a kisspeptin analog led to lower levels of the hormone FSH. Subacute treatment of a kisspeptin analog has been shown to reduce FSH levels, suggesting that this approach may be effective for controlling puberty onset.[95]

Kisspeptin Antagonists

Administering drugs that block kisspeptin's effects on the hypothalamus, pituitary, and ovary was a key step in elucidating the hormone's physiological role. The administration of peptide-234, a potent kisspeptin antagonist, decreases the mean GnRH concentration and inhibits spontaneous GnRH pulses in Rhesus monkeys. It was also shown to reduce LH pulses in ovariectomized sheep.[96] Antagonists of kisspeptin have shown promise and may find use in clinical practice. The most obvious use is in the treatment of patients with polycystic ovary syndrome (PCOS), postmenopausal symptoms, or early onset puberty,[97] all of which are associated with elevated LH concentrations. Peptide-234 infusions have been demonstrated to suppress reproductive organ growth in reproductive-age female rats. Even though there was no impact on BMI, the onset of pubertal milestones, such vaginal opening was also delayed.[98] Kisspeptin and neurokinin B antagonists may normalize GnRH production, lowering LH concentrations in PCOS women. In turn, this would boost oocyte maturation and revive folliculogenesis. GnRH and LH pulses are both elevated

in postmenopausal and PCOS women. Therefore, a kisspeptin antagonist may be helpful for women experiencing vasomotor symptoms during and after the menopause transition.[99] Currently, GnRH analogs are the sole medicine used to treat premature puberty. Mutations in the KISS1 and KISS1R genes have been linked to early-onset puberty.[100] Inhibiting pubertal growth using kisspeptin antagonists has been demonstrated in animal models of precocious puberty. The growing body of evidence and increasing understanding of kisspeptin's actions suggest that it may one day serve as a therapy alternative for this population of patients.

Conclusion

The growing body of evidence implicating kisspeptin as a crucial factor in aiding the start of puberty and establishing reproductive functions in animals strongly shows that kisspeptin plays a fundamental role in reproductive regulation. Additionally, it is essential in controlling the hypothalamic-pituitary-gonadal axis. Activities such as steroidogenesis, follicular development, ovulation, and ovarian senescence are all influenced by kisspeptin activity. For many people with infertility, the peptide hormone kisspeptin represents a ray of hope.

Competing Interests

The author declared that there are no competing interests.

Acknowledgments

This publication was supported by the Deanship of Scientific Research at Prince Sattam bin Abdulaziz University.

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International Journal of Biomedicine 13(4) (2023) 207-212
http://dx.doi.org/10.21103/Article13(4)_RA2

REVIEW ARTICLE

INTERNATIONAL JOURNAL OF BIOMEDICINE

Diagnostic Reference Levels in Pediatric Cardiac CT Imaging: A Literature Review

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Abstract

Background: Children are more sensitive to ionizing radiation than adults, with tissue sensitivity inversely proportional to age. The high sensitivity is due to their long life expectancy and rapidly dividing cells. Cardiac computed tomography (CCT) exposes patients to high doses of radiation, compared to other conventional examinations. Diagnostic reference levels (DRLs) were introduced to reduce unnecessary radiation exposure while maintaining image quality. This study intended to review the current literature regarding pediatric radiation dose during CCT examination and assess the role of DRL in patients' dose reduction.

Methods and Results: This review includes articles published on PubMed and Google Scholar between 2013 and 2022. Articles were screened to ensure their suitability for the review purpose of establishing the DRLs and the methods used. Five articles that include both simulated and actual relevant data were reviewed. Doses during CCT ranged from 0.2 mSv to 28 mSv depending on the type of procedure, patient's age and weight, scan length, and imaging protocol. This wide range showed that pediatric doses are not yet optimized, although studies follow guidelines established for pediatric DRLs. Similar studies need to be conducted to audit and renew pediatric DRLs. **(International Journal of Biomedicine. 2023;13(4):207-212.)**

Keywords: computed tomography • cardiac computed tomography • pediatric diagnostic reference levels

For citation: Khafaji MA, Albahiti SK. Diagnostic Reference Levels in Pediatric Cardiac CT Imaging: A Literature Review. International Journal of Biomedicine. 2023;13(4):207-212. doi:10.21103/Article13(4)_RA2

Abbreviations

CT, computed tomography; **CCT**, cardiac CT; **DRL**, diagnostic reference levels; **CTDIvol**, volume CT dose index; **DLP**, dose-length product; **DAP**, dose area product; **ICRP**, International Commission on Radiological Protection; **IAEA**, International Atomic Energy Agency.

Introduction

Computed tomography (CT) represents the main source of medical radiation to the general population.(1) Recent reports claimed that CT contributes 65% to 68% of the collective dose to patients from medical radiation, depending on the healthcare system level.(2) The frequency of the doses increased annually by 5% to 10%.(3) Cardiac CT (CCT) procedures expose the patient to a wide range of effective doses.(4,5) Children are more sensitive to ionizing radiation than adults, with tissue sensitivity inversely proportional to age. The high sensitivity is due to their long life expectancy and rapidly dividing cells. Recent studies showed that almost 10% of radiographic examinations are carried out on children.(6) CCT procedures exposed the patients to a higher dose than conventional examinations. With increasing the frequency

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of CCT procedures, reduction of radiation dose to patients by proper justification and optimization is recommended to ensure that patients' scans are acquired with minimal radiation dose while maintaining the image quality.[7]

The International Commission on Radiological Protection (ICRP) introduced the concept of Diagnostic Reference Levels (DRLs) in the 1990s[8] as a helpful tool for optimizing radiation doses in diagnostic and interventional radiology and nuclear medicine. DRLs are primarily used as investigation levels to assist in finding situations when dosage levels are extremely high. When DRLs are routinely exceeded, a local review is initiated to evaluate and justify the exposure. It is significant to emphasize that DRLs do not reflect dosage limitations or limits and are not designed for regulatory or commercial objectives.[9] The establishment, continuous evaluation, and usage of DRLs in every Member State in Europe have all been explicitly mandated since 1997.[10] DRLs can be established at local or national levels. The local DRL is defined as a reference level for an imaging procedure set in healthcare facilities within a part of a country, while the national DRL is a reference value set in a country based on data from a representative sample of healthcare facilities in that country. Local and National DRLs are defined for a specific clinical task and are based on the 75th percentile value of the distribution of the appropriate DRL quantity in a reasonable number of x-ray rooms and on the distribution of the median values of the appropriate DRL quantity observed at each healthcare facility, respectively.[11,12]

In the early 2000s, efforts were made to establish DRLs specifically for pediatric CCT imaging.[11] These efforts involved collaborations between healthcare professionals, medical physicists, and regulatory bodies. Data collection initiatives were launched to gather radiation dose information from a representative sample of pediatric patients undergoing CCT scans. Analyzing the collected data, researchers sought to identify trends and patterns in radiation doses. Dose distributions and reference levels that represented typical radiation doses in pediatric CCT imaging were determined.[12] These reference levels were aimed at optimizing radiation doses while maintaining diagnostic image quality. The establishment of pediatric-specific DRLs in CCT imaging was an iterative process. The calculated reference levels were compared with similar data from other institutions and national/international guidelines to ensure consistency and adherence to accepted standards and practices. Regular feedback and collaboration among healthcare professionals, medical physicists, and regulatory bodies were essential for refining and updating the DRLs. Over time, advancements in technology, changes in imaging practices, and evolving dose-reduction strategies necessitated the continuous review and updating of pediatric CCT DRLs.

The United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR) believes that children have a three to four times higher chance of acquiring cancer from radiation exposure than adults. Children also exhibit heightened sensitivity to certain types of radiogenic cancers, such as thyroid cancer, leukemia, breast cancer, and brain cancer, accounting for approximately 25% of the 23 types of radiogenic cancers identified.[13] It is important to note that children experience reduced shielding effects from adjacent organs, compared to adults, primarily due to their smaller body diameter.[14] Consequently, during diagnostic medical procedures, children may receive higher effective doses of radiation than adults undergoing the same procedure.[14,15] However, the implementation of pediatric-specific radiographic techniques has demonstrated the potential to reduce patient doses by approximately 90% significantly.[16] For those factors, it is crucial to develop exposure protocols that are specifically tailored to children, considering their age, size, relevant anatomy, and clinical indications during diagnostic radiological procedures. Such child-oriented protocols are essential for optimizing radiation safety and minimizing the potential risks associated with pediatric medical imaging.

The dosage descriptors used in creating images in CT by DRLs are the volume CT dose index CTDIvol and dose-length product (DLP). These dose descriptors, such as CTDIvol and DLP, are crucial in optimizing CT scans. However, it is important to note that they reflect the output of the CT scanner and do not directly estimate the radiation dose received by the patient. Nonetheless, they provide valuable metrics for quantifying radiation exposure during CT examinations.[17] There is a considerable variation in the radiation doses used in pediatric imaging, which can be attributed to the diverse body sizes of children.[18] While some degree of variation may be allowed, it is essential to ensure that the potential dangers of extra radiation exposure do not outweigh any dose disparities brought on by poor procedures and abilities. To find instances of excessively high radiation exposures, surveys have been carried out since 1950.[19,20] The primary goal of these early surveys was to offer suggestions for diagnostic x-ray methods. A significant challenge in comparing dose levels across different studies was the lack of standardized definitions, with varying terms such as exposure guides, guideline doses, guidance levels, and reference doses being used, making a comprehensive comparison difficult. As a consequence, the ICRP invented the term DRL in 1996, allowing for exact dose comparisons between treatments and a standardized approach. A guideline for developing DRLs was then established to further extend this notion and give optimization for a number of diagnostic medical tests.[16,19]

The DRL is a useful tool for maximizing patient safety by identifying increased dose levels that would not be justified based on picture-quality standards, according to the International Radiological Safety Commission.[17] It is significant to emphasize that, regardless of age or gender, the administration of DRLs is pertinent for all patients undergoing radio diagnostic procedures; DRLs are not meant to create dosage restrictions for particular individuals.[16,20]

The main goal of published DRL standards was to create reference values for radiological procedures.[21] Specific pediatric recommendations were required due to the growing concern about radiation dangers to children. As part of their human health series, the IAEA issued the first pediatric DRL standards in 2013.[22] This was a significant step forward in addressing the special concerns and requirements for optimizing

radiation protection in pediatric patients. In 2017, the ICRP released updated guidelines that included a specific subsection on pediatric DRLs.[16] The most comprehensive and current guidelines for pediatric DRLs were subsequently published in 2018 by the European Commission (EC).[23] These EC guidelines serve as the primary devoted reference for developing pediatric DRLs. The International Atomic Energy Agency (IAE) also provides valuable information on various aspects of pediatric DRLs under the RPOPs section.[24] These guidelines particularly emphasize the importance of establishing DRLs for modalities such as CT and fluoroscopy, which involve comparatively higher radiation doses. Numerous nations have proactively established DRLs for pediatric radiological procedures, with an emphasis on CT, in response to these recommendations.[25] Over the last ten years, the approach of creating pediatric DRLs has been increasingly popular in the field of radiation protection. As a result, we critically assess the pertinent literature in this study that relates to the creation of pediatric DRLs specifically for CCT procedures. The study also highlights the most popular methods used in this respect and contrasts them with the existing protocols.

Methods

This review includes articles published on PubMed and Google Scholar between 2013 and 2022. The search terms were "pediatric computed tomography diagnostic reference levels," "paediatric computed tomography diagnostic reference levels," "cellular radiosensitivity," and "cardiac computed tomography." Articles were screened to ensure their suitability for the review purpose of establishing the DRLs and the methods used. Five articles that include both simulated and actual relevant data were reviewed.[6,8,9,18,25]

Results

Although the concept of clinical indication-based DRLs (DRLci) was introduced by the ICRP in 2017,[26] the bulk of DRLs were developed using anatomical sites as a foundation. However, there are drawbacks to this strategy when it comes to CT. The same anatomical region may have different clinical reasons in CT, each requiring a separate set of exposure levels and methods. For instance, a chest CT might be used to assess the presence of lung cancer, pulmonary embolism, or coronary calcium scoring. These indications each call for particular scan settings and picture-quality requirements, underlining the requirement for unique DRLs for each clinical indication.[27] Additionally, as stated in action Number 2 of the EuroSafe Imaging Call for Action 2018 from the European Society of Radiology (ESR),[28] pediatric patients require special attention and consideration in medical examinations and procedures due to their increased vulnerability to the detrimental effects of radiation. The risk is partly due to their faster cell turnover and longer life expectancy, compared to adults.[29]

Table 1 presents the European DRLs according to European Guidelines (2018). In this table, the recommended age groups and weight groups for body examinations have been used.[30]

Table 1.

European DRLs for Thorax CT[30]

Weight	DRL	
	DLP (mGy.cm)	CTDIvol (mGy)
<5 kg	35	1.4
5-<15 kg	50	1.8
15-< 30 kg	70	2.7
30-<50 kg	115	3.7
50-<80 kg	200	5.4

DRL dose descriptors

Recent innovations in the development of DRLs in pediatric CCT imaging have focused on leveraging advancements in technology and dose-reduction strategies. Here are some notable innovations:

- Size-Specific Dose Estimates (SSDE) is a method that takes into account the patient's size, typically represented by the water-equivalent diameter, to estimate the patient-specific radiation dose. This approach provides a more accurate assessment of radiation dose than do traditional dose metrics. Implementing SSDE in the establishment of DRLs allows for more tailored dose optimization strategies in pediatric CCT imaging.[31]
- Organ-based dose modulation techniques adjust the radiation dose based on the anatomy and specific diagnostic requirements of the CCT examination. By optimizing the radiation dose distribution within the patient, organ-based dose modulation techniques can reduce unnecessary radiation exposure to sensitive organs, thus minimizing potential long-term risks in pediatric patients.[32]
- Iterative reconstruction algorithms have shown promise in reducing image noise and improving image quality in CT imaging. By using iterative reconstruction techniques, lower radiation doses can be employed while maintaining adequate image quality. Incorporating these techniques in the establishment of DRLs allows for dose reduction strategies that optimize both radiation exposure and diagnostic image quality in pediatric CCT imaging.[33]
- Artificial intelligence and machine learning algorithms have the potential to optimize radiation dose in pediatric CCT imaging. These technologies can analyze large datasets, including patient characteristics, imaging parameters, and radiation dose levels, to identify patterns and develop predictive models. By leveraging artificial intelligence and machine learning, DRLs can be refined and updated based on real-time data, leading to more precise and personalized dose optimization strategies.[34]

International collaborations and guidelines have played a crucial role in advancing the establishment of DRLs in pediatric CCT imaging. Guidelines and suggestions for radiation dose optimization have been made by groups like the ICRP and the IAEA. Collaboration among experts from different countries and institutions allows for the sharing

of best practices and the development of consensus-based approaches in establishing DRLs.

These recent innovations aim to enhance the optimization of radiation doses in pediatric CCT imaging by tailoring the dose to individual patient characteristics, leveraging advanced reconstruction techniques, harnessing the power of AI and machine learning, and promoting international collaboration and guidelines. By integrating these innovations into the establishment of DRLs, healthcare professionals can further improve patient safety and ensure the best possible diagnostic outcomes for pediatric CCT imaging. DRLs are dose benchmarks that help ensure that radiation doses in medical imaging procedures are optimized and kept as low as reasonably achievable while maintaining adequate image quality for accurate diagnosis. DRLs provide guidance to healthcare professionals regarding acceptable radiation dose ranges for specific procedures or patient groups.

Establishing DRLs

Establishing DRLs for pediatric CCT imaging involves several steps:[35,36]

• Data Collection: Radiation dose data from a representative sample of pediatric patients undergoing CCT scans are collected. This data includes patient characteristics (age, weight, height), scanning parameters (tube voltage, tube current, scan length), and dose metrics (such as DLR or effective dose).

• Data Analysis: The collected dose data is analyzed to identify trends and patterns in radiation doses. Statistical methods are used to calculate dose distributions and identify reference levels that represent typical radiation doses for pediatric CCT imaging.

• Peer Comparison: The calculated reference levels are compared with similar data from other institutions or national/international guidelines. This helps ensure that the established DRLs are consistent with accepted standards and practices.

• Iterative Process: DRLs are not fixed values but should be regularly reviewed and updated based on advancements in technology, changes in imaging practices, and evolving dose-reduction strategies. Regular feedback and collaboration among healthcare professionals, medical physicists, and regulatory bodies are essential for maintaining relevant and effective DRLs.

Table 2.

Diagnostic Reference Levels and Effective Dose in Pediatric CCT.[36]

Weight (kg)	Age (years)	Cardiac Diseases	DAP ($Gy \cdot cm^2$)	Effective Dose (mSv)
3- 5	<1	Atrial septal defect	35	1.8
10 -15	1-3	Pulmonary valve stenosis	40	2.5
20-25	3 – 5	Coarctation of the aorta	50	3.7
30-35	5 -10	Ventricular septal defect	60	4.3
40-45	10- 15	Patent ductus arteriosus	75	6.2
50-55	>15	Coronary artery disease	100	8.9

The establishment of DRLs in pediatric CCT imaging aims to promote radiation safety and optimize imaging practices for children. By adhering to these reference levels, healthcare providers can ensure that radiation doses are minimized while maintaining diagnostic image quality, thus reducing potential long-term radiation risks for pediatric patients. It's worth noting that specific guidelines and protocols for establishing DRLs may vary between countries and institutions, and local expertise in medical physics is crucial in this process to ensure accurate data collection, analysis, and interpretation.[32] By establishing reference levels for radiation doses in pediatric CCT imaging, healthcare professionals can strive to achieve the necessary diagnostic information while minimizing radiation exposure. DRLs provide a valuable guide for healthcare providers, enabling them to optimize radiation doses and adhere to the principle of keeping doses as low as reasonably achievable (ALARA) while maintaining diagnostic image quality. This ensures that the radiation doses administered during the imaging procedure are tailored to the individual needs of pediatric patients, promoting the best possible clinical outcomes.

Establishing the DRL in pediatric CCT imaging is of utmost importance in ensuring optimal radiation dose levels for accurate diagnosis while prioritizing patient safety. The unique characteristics of pediatric patients, such as their higher sensitivity to radiation and longer life expectancy, require tailored approaches in dose optimization. By implementing DRLs, healthcare professionals can monitor and control radiation doses, minimizing unnecessary exposure and potential risks. The involvement of international organizations like the ICRP, and the IAEA is crucial in developing guidelines, recommendations, and regulatory frameworks to guide pediatric CCT imaging practices. These organizations contribute to the advancement of radiation safety, provide technical assistance, and promote best practices in dose optimization and quality assurance.

Although research and literature on DRLs in pediatric CCT imaging is limited, it has emphasized the need to consider factors such as patient age, size, clinical indication, and anatomical considerations when determining appropriate dose levels. The literature also highlights the importance of collaboration between healthcare professionals, radiologists, medical physicists, and regulatory bodies to continuously improve dose optimization strategies and enhance patient care. By implementing exposure protocols and adhering to established DRLs, healthcare providers can balance between obtaining high-quality diagnostic images and minimizing radiation risks in pediatric CCT imaging. Ongoing research and advancements in this field will further contribute to the refinement of DRLs and ensure continued improvements in patient safety and outcomes (Tables 2 and 3). Data concerning the pediatric CCT DRLs in the literature are limited; there are countries with no DRLs or lack of DRL values for some age/weight groups. The manufacturers should facilitate the procedure for establishing DRLs for cardiac examination in terms of dose quantities and units. Updated values of DRLs should take into consideration modern technology and practices and follow guidelines both for imaging and for establishing

DRLs. In addition, there is a need to establish DRLs based on clinical indications, which could be achieved through collaboration between the physician, medical physicist, and radiographers.

Table 3.

Diagnostic Reference Levels (DRLs) for Pediatric CCT Imaging (Hypothetical)

Weight (kg)	Age (years)	CTDIvol (mGy)
5 -10	1-3	2.5
15-20	4 -7	3
25-30	8 -12	3.5
35-40	13-18	4
45-50	>18	4.5

Declaration

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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International Journal of Biomedicine 13(4) (2023) 213-220
http://dx.doi.org/10.21103/Article13(4)_RA3

REVIEW ARTICLE

INTERNATIONAL JOURNAL OF BIOMEDICINE

Platelet-Rich Fibrin in Oral Surgery and Endodontic Procedures as a Regenerative Biomaterial: A Review Article

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Abstract

Background: One of the major challenges in clinical research is to incorporate materials and procedures into dentistry that can improve clinical outcomes, increasing percentages of success. Platelet-rich fibrin (PRF) is a surgical, biological additive prepared by manipulation of autologous blood, which stimulates and regulates inflammation during and after oral surgery. This study aims to assess the potential benefits of using PRF in modern oral and endodontic surgery to accelerate radiographic healing and reduce postoperative discomfort.

Methods and Results: Electronic literature research was conducted using the biomedical search engine "National Library of Medicine," PubMed/MEDLINE database from March 2000 to June 2023. To obtain results that involved the whole scope of dentistry and PRF, the keywords used for the search were "PRF," "PRF in dentistry," and "PRF on apical surgery." The articles were selected by reviewing the titles and abstracts of the articles with the word "platelet-rich fibrin (PRF)." Articles related only to clinical applications in general dentistry and its different application fields were hand-searched in applicable, significant journals, and reference lists of included studies were broadcast with no language limitation. The inclusion criteria set for this review were as follows: all case reports, case series, original research papers, review papers, in vitro/in vivo studies, animal studies, and controlled clinical trials on PRF used in dentistry-related studies.

Current studies, in vitro and in vivo, have confirmed safe and encouraging results, without opposing outcomes, related to the use of PRF alone or in a mixture with other biomaterials. The use of PRF treatment has been shown to enhance and promote natural tissue healing support.**(International Journal of Biomedicine. 2023;13(4):213-220.)**

Keywords: platelet-rich fibrin • endodontic procedures • apical surgery

For citation: Morina LL, Muratovska I, Hajdari B, Agani Z, Leci B, Bimbashi V. Platelet-Rich Fibrin in Oral Surgery and Endodontic Procedures as a Regenerative Biomaterial: A Review Article. International Journal of Biomedicine. 2023;13(4):213-220. doi:10.21103/Article13(4)_RA3

Abbreviations

MTA, mineral trioxide aggregate; **PRF**, platelet-rich fibrin; **i-PRF**, injectable PRF; **PRP**, platelet-rich plasma; **PRGF**, plasma rich in growth factors.

Introduction

One of the major challenges in clinical research is to incorporate materials and procedures into dentistry that can improve clinical outcomes, increasing percentages of success.[1] Platelet-rich fibrin (PRF) is a surgical, biological additive prepared by manipulation of autologous blood, which stimulates and regulates inflammation during and after oral surgery.[2] As a biomaterial, it appears to provide natural and satisfactory alternative results and low risks. The possibility of accelerating bone regeneration in periapical and other surgical defects might be of great interest to clinicians to proceed with soft and hard tissue regeneration for complete healing after oral surgery.

Based on a literature review, PRF is a very important factor that promotes and regulates inflammation during and after oral surgery.[1,2] PRF, which is emerging as a biological revolution in dentistry, is an immune and platelet concentrate with specific composition, three-dimensional architecture, and associated biology that collects all the constituents of a blood sample to favor wound healing and immunity.[3]

This study aims to assess the potential benefits of using PRF in modern oral and endodontic surgery to accelerate radiographic healing and reduce postoperative discomfort.

Materials and Methods

Electronic literature research was conducted using the biomedical search engine "National Library of Medicine," PubMed/MEDLINE database from March 2000 to June 2023. To obtain results that involved the whole scope of dentistry and PRF, the keywords used for the search were "PRF," "PRF in dentistry," and "PRF on apical surgery."

The articles were selected by reviewing the titles and abstracts of the articles with the word "platelet-rich fibrin (PRF)." Articles related only to clinical applications in general dentistry and its different application fields were hand-searched in applicable, significant journals, and reference lists of included studies were broadcast with no language limitation. The inclusion criteria set for this review were as follows: all case reports, case series, original research papers, review papers, in vitro/in vivo studies, animal studies, and controlled clinical trials on PRF used in dentistry-related studies.

Platelet Concentrate Evolution

The regenerative potential of platelets was reported in the 1970s, and hematologists created the term PRP to describe plasma with a platelet count above that of peripheral blood, which was initially used as a transfusion product to treat patients with thrombocytopenia.[4,5]

In the 1980s, fibrin glue was the first blood-related product used in surgery, and at that time, fibrin glue was used as a hemostatic agent and surgical glue. Many authors then stated that it was used because of its positive effects on tissue healing.[6]

Platelet-rich plasma (PRP) was available during the 1990s, with the rapid development of techniques and equipment. In oral surgery, the first PRP study was introduced by Whitman et al. in 1997.[7] Later, at the beginning of the 2000s, PRF was presented for endodontic surgery as an autologous fibrin with a large quantity of platelets and leukocyte cytokines. This product can be used to improve wound healing in immune-compromised and diabetic patients. Additionally, as PRF stimulates coagulation and wound closure, it can be used as an adjuvant in patients on anticoagulant therapy.[8]

The PRF concept, as described in France by J. Choukroun in 2001,[9] is a second-generation platelet concentrate used for its ability to improve tissue repair and regeneration. It was presented as a replacement for PRP, which is known as a first-generation platelet derivative, as it is simpler and safer than PRP.[1] Choukroun et al. reported that platelets and leukocyte cytokines are important in the key role performance of this biomaterial; nonetheless, the fibrin matrix supporting them was very helpful in constituting the determining elements responsible for the real therapeutic potential of PRF.[1,9] Choukroun's PRF is a biomaterial used by itself and as an adjunct to grafts. It has been successful as it delivers high doses of growth factors and has anti-inflammatory properties. PRF is of great promise in dentistry, ranging from implantology, sinus lift procedures, and treatment of endodontic and periodontal lesions to regeneration of necrotic pulp. PRF is used to fill bony defects after periapical surgeries such as root-end resection.[10]

Protocol for PRF Preparation

According to Choukroun's original protocol that tries to collect platelets and the released cytokines in a fibrin clot, the PRF protocol requires only centrifuged blood without any addition of anticoagulant and bovine thrombin. Formerly, 10ml of whole blood sample was taken without anticoagulant in a 10 mL glass or glass-coated plastic tube and then immediately centrifuged at 3,000 rpm for 10 minutes.[9,10] The protocol tries to accumulate platelets and the released cytokines in a fibrin clot.[11] The plasma machine for preparation was a T-Lab Centrifuge (S-106) PRP, PRF Swing Rotor. Then, the blood sample settles into three layers: a straw-colored fraction of acellular platelet-poor plasma at the peak level, a PRF clot at an intermediate level, and a red fraction of red blood cells at the base level. Driving out the fluids trapped in the fibrin matrix by squeezing the PRF clot between pieces of sterile dry gauze, medical practitioners will obtain a highly resistant, autologous PRF membrane (a highly promising biomaterial) for multiple clinical usages.[12]

Advantages of the Clinical Application of PRF

PRF is autologous, derived from the patient's own blood, and is safe and fast. Therefore, disease transmission is not an issue, and PRF technology is readily available. It is easy to prepare and use, and it can be produced immediately by a chairside procedure. Currently, it is widely applicable in dentistry and financially realistic for both the patient and the clinician. The most important advantage is that there is no risk of a rejection reaction (no foreign body response). It provides the surgical wound area or defect not only with a matrix or scaffold permitting cell migration into the defect area, but also crucial biological signals or growth factors that can accelerate the wound healing and regeneration process.[12,13]

Benefits of PRF Compared to PRP and PRGF

The benefits of PRF are that it is simpler to produce and does not require blood manipulation: no additives, no

anticoagulants, no animal thrombin or calcium chloride, unlike PRP and PRGF. PRF produces a larger share of blood product over the share of blood taken that contains more healing factors, more stem cells, and less trauma. The benefits of PRF treatment in wound and bone healing, its antibacterial and antihemorrhagic effects, the low risks with its use, and the availability of easy and low-cost preparation methods encourage more clinicians to adopt this technology to benefit their patients.[12,13]

PRF has wide applicability, from dentistry to medicine, with excellent results in the short term. All studies have shown its safety in maxillofacial applications. Recently, much research has been done on PRF, and numerous cases have been reported regarding the use of PRF clots and PRF membranes. Most research has focused on using PRF in oral surgery for bone augmentation, sinus lifts, and avulsion sockets;[13-15] and in periodontics to correct intrabony defects, gingival recession, guided bone regeneration, and periapical lesions. Some case reports have shown that a combination of PRF gel, hydroxyapatite graft, and guided tissue regeneration plays a very important role during endodontic procedures for regeneration in open apex, regenerative pulpotomies, and periapical surgeries.[14-17] Platelet concentrates have been used extensively in oral and craniofacial interventions for hard tissue regeneration and soft tissue healing. In addition, these concentrates could decrease inflammatory complications, such as pain and swelling due to the inhibition of cytokine secretion.[18]

Platelet concentrates today are used in many orofacial disciplines, such as endodontic regeneration, osteoradionecrosis, closure of oro-antral communication, oral ulcers, and temporomandibular disorders.[19]

Endodontic Procedures

Endodontic failures are the most common reason periapical lesions are caused, and the main reason is the persistence of bacteria (intracanal and extracanal). Studies have shown that as the size of the lesion increases, so does the risk of root canal therapy failure after treatment.[20]

This increased risk is probable because as the size increases, so does the probability that the lesion may have evolved to be cystic by nature.[21] Infection, inflammation, and bone resorption are closely related to apical periodontitis development. Biochemical mediators are released locally to stimulate the immune response during inflammatory events.[22] The integrity of bone tissues depends on maintaining a delicate equilibrium between bone resorption by osteoclasts and bone deposition by osteoblasts.[23] With advances in surgical techniques, the outcome of surgical endodontic treatment appears to be more promising and predictable than before. Apical surgery is now considered a predictable treatment option to save a tooth with apical pathology that cannot be managed by conventional, nonsurgical endodontic procedures.[24]

Different natural materials have been used: bone grafts platelet-rich plasma (PRP), bone morphogenic proteins (BMPs), platelet-derived growth factor (PDGF), parathyroid hormone, and enamel matrix protein derivative (EMD) which have been locally applied to promote the healing potential of the surgical site.[25] Therefore, PRF can be used in a common form with mineral trioxide aggregate (MTA) as an alternative for creating artificial root-end barriers and to induce faster periapical healing in cases with large periapical lesions.[15]

The use of PRF in the form of a membrane can prevent the extrusion of material. PRF can also be used in regenerative pulpotomy procedures, in which the coronal pulp is removed, and the pulp wound is covered with PRF and then sealed with MTA and glass ionomer cement. Clinical data have shown that the healing outcome of apical surgery in teeth with permanent restorations is better than in teeth with temporary restorations.[26,28]

Regenerative Endodontic Procedures

In certain aspects of surgical endodontics, such as the treatment of periapical lesions and the regeneration of pulp (in the case of a tooth with a previously necrotic pulp with an open apex), PRF could be used as an ideal scaffold material for the repair and regeneration of the tissue since it acts as a matrix for the migration of cells.[28-30]

PRF also promotes the revascularization of teeth by releasing growth factors. The three criteria for the success of any regenerative procedures, including regenerative endodontics, are stem cells, signaling molecules, and an ideal scaffold. A study conducted by Huang found that there was a proliferation of human dental pulp cells and increased protein expression of osteoprogenin and alkaline phosphatase activity in cases where PRF was used.[31]

Shivashankar et al.[32] reported a case of revitalization of a tooth with necrotic pulp and an open apex, where PRF was the biomaterial he used. Once the inflammation subsides, the dental pulp cells differentiate into odontoblast-like cells under the influence of Hertwig's epithelial root sheath. It was reported that revitalization of a necrotic infected immature tooth was possible when PRF was used as a biomaterial for regenerating the pulp-dentin complex under conditions of total canal disinfection.[32]

Based on the literature, the difference between natural blood clots and PRF is that the latter is more homogeneous and stable, and is easier to handle and place in the indicated local area. PRF can be used as a scaffold in the regenerative endodontic treatment of traumatized immature nonvital teeth.[33] Slow polymerization during PRF preparation generates a fibrin complex similar to the natural linkage (in vitro). Unlike the other platelet concentrates, PRF can progressively release cytokines during fibrin matrix remodeling.[34]

Six studies[15,32,35-38] present a total of 55 patients who were treated with PRF by placing it into the dental canals or in the periradicular lesions. One study reported five patients with incomplete healing. Two patients did not respond to treatment;[38] in the remaining 48 patients, complete resolution and bone regeneration of the apical lesions were achieved. The treated pathologies were immature teeth with necrotic pulps, acute chronic apical abscess, and suppurated chronic apical periodontitis.

Recently, Lin et al.[39] demonstrated that the healing of periapical tissues is a "programmed event." More than the size of the lesion, it is the microenvironment consisting of the progenitor/stem cells, extracellular matrix, and bioactive molecules that play a crucial role in tissue regeneration or scar formation during wound healing.

One potential method to improve the disinfection performance is by using atmospheric-pressure cold plasmas.[39-41]

However, due to the shape of the narrow channel geometry of a root canal, which typically has a length of a few centimeters and a diameter of one millimeter or less, for better efficacy in killing bacteria, plasma should be generated inside the root canal. Hereafter, when plasma is generated inside the root canal, all kinds of reactive agents, including short-life pieces, such as charge particles, could play some roles in the death of bacteria. Lu et al. used a reliable and user-friendly plasma-jet device that could generate plasma inside the root canal.[42]

Another study by Hiremath et al.[43] described and reported affirmative results with pulpotomy using PRF. The effectiveness of this method must be demonstrated in long-term trials with larger sample sizes to justify its use in treating pulpitis. Pulpotomy with PRF could be a substitute treatment for MTA or other materials.

Bains et al.[44] reported the applicability of PRF for managing iatrogenic perforation of the pulpal floor in the furcation region of the first mandibular molar; PRF and MTA appeared to be most favorable materials for good long-term clinical results. Regenerative endodontics has been used in different root canal procedures, including apexification, apexogenesis, pulpotomy, and endodontic apical surgery.[45]

In recent studies, researchers have enhanced periapical bone regeneration, root development, and pulp vitality. In addition, a comprehensive, systematic review of clinical evidence showed that applying PRF is a successful procedure in treating immature teeth. Although the level of evidence was weak, reports were included, and further well-designed studies with longer follow-up periods are needed.[45]

The following brief literature review on the various applications of PRF in endodontic therapy considered a variety of methods that have been performed, such as mechanical cleaning, irrigation, laser irradiation, ultrasound, and application of hypochlorite and other antibacterial compounds. Regenerative endodontic procedures are widely being added to the current armamentarium of pulp therapy procedures.[31,46-50] These biologically based procedures are designed to restore the function of a damaged and nonfunctioning pulp by stimulation of existing dental pulp stem and progenitor cells present in the root canal under conditions that are favorable to their differentiation.[47,48]

Recent case reports have shown that the combined use of PRF and MTA as root-filling material is beneficial for the endodontic management of an open apex.[51,52]

Revascularization and Revitalization

Revascularization is the most studied and successful approach to regenerative endodontics.[51] Revitalization of necrotic infected immature teeth is possible under conditions of total canal disinfection combined with the additive effect of PRF.[52,53] PRF is proposed as an ideal biomaterial for regenerating the pulp-dentin complex because it is a potentially valid scaffold material containing leukocytes and growth factors to facilitate tissue healing and regeneration in immature necrotic teeth in children.[51-53] The repair and regenerative potential of PRF and enhanced cellular metabolism with laser biostimulation, in combination with the sealing ability of MTA, enhance the clinical success in pulpotomy and apexification procedures.[51] Revitalization, revascularization, and regenerative pulp therapies still need to be validated with robust clinical trials.[52,53]

Endodontic Surgery (apical surgery)

PRF clots (gels) serve as an ideal scaffold in root-end surgical procedures to enhance soft tissue healing and bone regeneration.[15,54,55] Other researchers have reported that PRF may not necessarily improve the outcome of treatment.[56]

It has been suggested that the combination of PRF and β-TCP for bone augmentation in the treatment of periapical defects is also more effective at increasing healing time than using bone substitute material alone.[57] PRF combined with an alloplastic bone substitute has been successfully used for managing combined endodontic-periodontal lesions.[58]

Oral and Maxillofacial Surgery

PRF represents a revolutionary step in the platelet gel therapeutic concept. Post-extraction socket augmentation and healing along with the filling of avulsion sockets with PRF lead to very favorable results when bone walls are intact.[57,58] A combination of PRF with bone substitutes and other adjuncts may be necessary for residual defects where one or several walls are missing or damaged to provide an adequate reconstruction of bone volume. PRF increases the cohesion between the graft materials, as fibrin acts as a physiological glue between the wound tissues. In cases of wide sockets and lesions where primary closure is difficult, PRF membranes can be used as a covering and protective membrane that promotes re-epithelialization of the site and accelerates the merging of the wound margins. The elasticity and strength of the PRF membrane make it easy to suture.[58-60]

The healing and remodeling of an extraction socket is highly dependent on the initial stabilization of the blood clot and quick closure of the gingival wound. This can be achieved by placing a fibrin plug in the socket and closing it with a fibrin membrane. PRF will act as a stable blood clot for neovascularization and accelerated tissue regeneration. PRF is recommended as a useful procedure to reduce the early adverse effects of inflammation, such as postoperative pain, and to promote soft tissue healing and bone regeneration processes.[60-64]

Clinical situations where post-extraction socket augmentation with PRF was specifically indicated for early or delayed implant placement, and immediate post-extraction implant placement improved the strength of bone integration to dental implants.[65-74]

PRF led to aggregate tissue regeneration, making healing more effective and capable in both hard and soft tissue. Using it to regenerate tissues can repair much damage caused by peri-implant recessions, and maxillary sinus lift helps in rapid healing by accelerating the bone integration of the implants.[75-79]

There are several advantages to using PRF, such as easy and simplified chairside preparation of PRF, cost-effectiveness, release of relatively constant concentrations of growth factors over a period of 7 days, and rapid and excellent healing of the periodontium. Many studies of PRF have found it to be more efficient and with fewer controversies about its final clinical results than PRP.[74]

Anilkumar et al.[80] reported on PRF as a potential novel root coverage approach for treating gingival recession in mandibular anterior teeth using a combined laterally positioned flap technique and PRF membrane. The major differences between PRP and PRF glues are attributable to the gelation mode. Fibrin and CPRP adhesives use bovine thrombin and calcium chloride association to initiate the last phase of coagulation and polymerization of sudden fibrin. The advantage of PRP is the release of significantly more proteins at earlier time points, whereas PRF displayed a continual and steady release of growth factors over a 10-day period. Moreover, in general, it was observed that the new formulation of PRF (A-PRF) released significantly higher total quantities of growth factors than traditional PRF. Based on these findings, PRP can be recommended for fast delivery of growth factors, whereas A-PRF is better suited for long-term release.[80] Another study favored PRF over PRP, and the results from the present study favored the use of naturally formulated i-PRF over traditional PRP with anticoagulants.[79,81]

Further investigation into the direct role of fibrin and leukocytes contained within i-PRF is therefore warranted to better elucidate their positive role in i-PRF on tissue wound healing. The results from the present study favored the use of naturally formulated i-PRF over traditional PRP with anticoagulants.[81]

Other Clinical Applications

PRF is used in different fields. It has been shown to produce as much as a 10-fold decrease in osteomyelitis infections.[82] To reduce post-extraction complications in medically compromised cases, PRF can be used to minimize post-extraction pain and bleeding, close oro-antral fistulas, manage oro-antral communications, etc. However, a split-mouth control study devoted to evaluating the efficacy of PRF on postoperative edema and pain after impacted mandibular third molar surgery has found that using or not using PRF to reduce postoperative pain and edema in third molar surgery were equally successful.[83-86]

Conclusion

Current studies, in vitro and in vivo, have confirmed safe and encouraging results, without opposing outcomes, related to the use of PRF alone or in a mixture with other biomaterials. It has several indications and advantages to be used in dentistry and medicine. Currently, the use of PRF treatment has been shown to enhance to enhance and promote natural tissue healing support.

This review article attempted to summarize the use of PRF accepted as a minimally invasive technique with low risk and satisfactory clinical results in oral and maxillofacial surgery.

Competing Interests

The authors declare that they have no competing interests.

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International Journal of Biomedicine 13(4) (2023) 221-227
http://dx.doi.org/10.21103/Article13(4)_RA4

REVIEW ARTICLE

INTERNATIONAL JOURNAL OF BIOMEDICINE

Oral Mucosal Changes in Acrylate Prosthesis Wearers Among Diabetic Patients: A Review Article

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Abstract

Diabetes mellitus (DM) is one of the significant health problems in the world. Diabetes affects most parts of the human organism, and the oral cavity is no exception. Among oral manifestations related to DM are dry mouth, tooth decay, periodontal disease, gingivitis, oral candidiasis, burning mouth syndrome, disorders of taste, oral lichen planus, geographic tongue, fissured tongue, delayed wound healing, increased incidence of infection, salivary dysfunction and neurosensory disorders. According to the literature, edentulous patients are more likely to have DM in comparison to the nondiabetic population. The effect of total prostheses on the oral mucosa (OM) has been the subject of many scientific studies. This study aimed to investigate existing evidence related to changes in the OM in DM patients who are wearers of total acrylic prostheses.

Based on the reviewed literature, changes in the OM are much more pronounced in DM patients wearing acrylate prostheses and are caused by severe inflammation and delayed keratinization. A total acrylate prosthesis introduces additional trauma, inflammation, and stress in DM patients. **(International Journal of Biomedicine. 2023;13(4):221-227.)**

Keywords: diabetes mellitus • acrylate prosthesis • oral mucosa • cytological changes

For citation: Kurshumliu ED, Kovacevska G, Shala K, Krasniqi BM, Kurshumliu F. Oral Mucosal Changes in Acrylate Prosthesis Wearers Among Diabetic Patients: A Review Article. International Journal of Biomedicine. 2023;13(4):221-227. doi:10.21103/Article13(4)_RA4

Abbreviations

BMS, burning mouth syndrome; **DM**, diabetes mellitus; **DS**, denture stomatitis; **OLP**, oral lichen planus; **OM**, oral mucosa; **T1DM**, type 1 DM; **T2DM**, type 2 DM.

Introduction

Diabetes mellitus (DM) is one of the significant health problems in the world. It is a metabolic disorder characterized by chronic hyperglycemia and related disturbances of fat and protein metabolism due to defects in insulin secretion, action, or both.[(1)]

Type 1 DM (T1DM), previously known as insulin-dependent DM or juvenile-onset diabetes, affects 5%-10% of all diagnosed cases of diabetes. Type 2 DM (T2DM) makes up the vast majority of cases – 80%-90%.[(2)]

Several pathogenic processes are involved in the development of diabetes. These range from autoimmune destruction of the β-cells of the pancreas with consequent

insulin deficiency to abnormalities resulting in insulin action resistance.

Diabetes affects most parts of the human organism, and the oral cavity is no exception.[3] Among oral manifestations related to DM are dry mouth, tooth decay, periodontal disease, gingivitis, oral candidiasis, burning mouth syndrome (BMS), disorders of taste, oral lichen planus (OLP), geographic tongue, fissured tongue, delayed wound healing, increased incidence of infection, salivary dysfunction and neurosensory disorders.[4]

The complete absence of teeth may be due to many factors, such as caries, periodontal pathologies, trauma, and oral cancer.[5] According to the literature, edentulous patients are 1.82 times more likely to have DM in comparison to the nondiabetic population.[6] Most edentulous patients are rehabilitated by application of total prostheses, which replace the lost bone and teeth. Complete dentures restore the function of the jaw-tooth system of the face. The effect of total prostheses on the oral mucosa (OM) has been the subject of many scientific studies.[1-47]When OM is covered by a denture, it is sandwiched between the denture and the underlying bone. Although protected from the direct effects of stimulation by food, it is subjected to the traumatic effect of the denture.[7] It is not surprising that there is no general agreement on the effects of dentures on OM since there are many factors that may alter them, such as the type of denture, the type of denture base material, the denture hygiene, the duration of denture wear each day, the length of denture exposure, the physical condition of the denture and dentition in the opposing arch.[7]

During mastication, the OM beneath the denture is critical in distributing occlusal loads to the underlying bony ridge over a large denture-supporting tissue interface. [8-10] Clinical assessment of the supporting tissue in complete denture patients is an important step in treatment planning, as mucosal displacement can disturb the denture balance. The dentist should adequately examine the mucosa, and the clinical scenario differs from patient to patient.[11]

The OM can be classified into lining mucosa, masticatory mucosa, and specialized mucosa. The masticatory mucosa is found on the gingiva and the hard palate. It has a keratinized and, in some areas, a parakeratinized stratified squamous epithelium. Parakeratinized epithelium is similar to keratinized epithelium except that the superficial cells do not lose their nuclei, and their cytoplasm does not stain intensely with eosin. The nuclei of the parakeratinized cells are pyknotic (highly condensed) and remain until the cells are exfoliated. The keratinized epithelium of the masticatory mucosa resembles that of the skin but lacks a stratum lucidum. The underlying lamina propria consists of a thick papillary layer of loose connective tissue that contains blood vessels and nerves; some send bare axon endings into the epithelium as sensory receptors, and some end in Meissner's corpuscules. Deep to the lamina propria is a reticular layer of denser connective tissue. As in the skin, the depth and number of connective tissue papillae contribute to the relative immobility of the masticatory mucosa, thus protecting it from frictional and shearing stress. At the midline of the hard palate, in the palatine raphe, the mucosa adheres firmly to the underlying bone. The reticular layer of the lamina propria blends with the periosteum; thus, there is no submucosa. The same is true of the gingiva. Where there is a submucosa underlying the lamina propria on the hard palate, it contains adipose tissue anteriorly (fatty zone) and mucous glands posteriorly (glandular area) that are continuous with those of the soft palate. In the submucosal regions, thick collagenous bands extend from the mucosa to the bone.[12]

Acrylic-based resins are frequently used in daily dental practice for prostheses as they can provide essential properties and have the necessary characteristics for their use in diverse functions. During the polymerization of these materials, the residual monomer is released, which may be cytotoxic to the OM. The released monomer depends on the modes of polymerization as well as on the degradation of the polymer under certain conditions that are present in the oral cavity.[13]

Since prostheses are generally foreign bodies in the oral cavity, mucosal lesions in their undersurface may be considered a rather normal occurrence. Their frequency is thought to be even greater in patients suffering from DM, considering the sensitivity of the OM in these patients.

Given all the oral manifestations in DM mentioned previously, the wearing of complete dentures in diabetic patients is complicated by the higher incidence and gravity of oral mucosal lesions compared to nondiabetic patients. Due to the complex nature of the disease, changes in the oral epithelium under total acrylic prostheses will be a clinical challenge in finding methods and treatments to manage and prevent these changes in patients with total acrylic prostheses suffering from DM. There are experimental studies in the literature designed to analyze the changes that occur under total prosthesis in patients with DM and the factors that may affect these patients.

This study aimed to investigate existing evidence related to changes in the OM in DM patients who are wearers of total acrylic prostheses.

Material and Methods

We reviewed published data on the role of Oral Mucosal Changes in Acrylate Prosthesis Wearers Among Diabetic Patients, searching through PubMed, MEDLINE and Scopus, using search terms with suitable keywords. The search terms were "diabetes mellitus," "oral mucosal changes," "acrylate prosthesis," and "mucosal cytological changes." Studies have been classified according to the year of publication, respective pathologies, number of cases, and conclusion.

Results

Our focus was on 26 articles published between 1982 and 2022 describing oral mucosal lesions in patients with diabetes, many of whom are total acrylate denture wearers.

Table 1 summarizes the findings in the analyzed articles. In 3 studies with 340 DM subjects with xerostomia, all had dry mouth sensitivity and decreased saliva.[15-17] In 2 studies involving 740 denture wearers, xerostomia had an adverse effect on oral functions.[14,19]

Table 1.

Summary of the reviewed articles.

Author(s)	Year of publication	Type of study	Pathology	No. of cases	Conclusion
Hoseini A, et al. [15]	2017	CCS	Xerostomia	150	Diabetic patients (T1DM and T2DM) revealed a lower salivary flow rate and higher xerostomia than healthy controls.
Eldarrat AH, et al. [16]	2011	QS	Xerostomia	100	Diabetic patients who do not carefully control their blood glucose levels will be at high risk of systemic and oral complications.
Cicmil A, et al. [17]	2020	CCS	Xerostomia	90	The findings have indicated that a decreased salivary flow rate could significantly impact oral health status in type 2 diabetics.
Al-Dwairi Z, et al. [14]	2014	QS	Xerostomia	455	Xerostomia adversely affects oral functions and overall satisfaction with dentures.
Aslam A. [19]	2017	QS	Xerostomia	285	Dry mouth appears to have a significant impact on the oral function in denture wearers.
Soell M, et al. [20]	2007	RA	Candidiasis	10 S	Diabetics have an increased predisposition to the manifestation of oral diseases like candidiasis, which is associated with poor glycemic control and therapeutic dentures.
Lotfi-Kamran MH, et al. [21]	2009	CCS	Candidiasis	92	Mycological findings from the study revealed that diabetes mellitus can increase colonization of Candida in denture and mouth
Webb BC, et al. [22]	1998	RA	Candidiasis	N\A	This study observed that the use of antifungal and antibacterial agents effectively reduces the number of Candida and aerobic bacteria from the prosthesis surface.
Ganapathy DM, et al. [23]	2013	POS	Candidiasis	15	A positive correlation was observed between oral candidiasis in complete denture-bearing mucosa and elevated blood glucose levels and oral hypoglycemic drug therapy has a positive effect in controlling oral Candida colonization in complete denture wearers with T2DM.
Cristina de Lima D, et al. [24]	2008	CCS	Oral mucosal lesion	60	No significant differences were observed in salivary flow, denture retention, or oral lesions in diabetic and nondiabetic subjects.
Trentin MS, et al. [25]	2017	CSS	Prosthetic stomatitis	250	Diabetic patients with complete upper denture presented with higher incidence of prosthetic stomatitis compared to non-diabetics
Khatibi M, et al. [26]	2015	CS	Prosthetic stomatitis	112	A significant relationship between T2DM and the prevalence of denture stomatitis was found.
Bookout GP, et al. [27]	2022	RA	BMS	N/A	The diagnosis and management of burning mouth syndrome are challenging and require a multidisciplinary approach - medical and psychological. The cause of the disorder remains unknown, and the treatment is empirical.
Aravindhan R, et al.[28]	2014	RA	BMS	N/A	The exact cause of BMS often is difficult to pinpoint and is probably of multifactorial origin and may be idiopathic.
Nada A, et al. [29]	2020	CSS	BMS	250	BMS in patients with diabetic neuropathy is complicated. The contributing oral factors are associated with poor metabolic control.
Mukatash-Nimri G, et al. [30]	2017	CCS	BMS	129	Significant positive associations were found between local factors (i.e., wearing complete dentures with unsatisfactory retention or jaw relationship, dry mouth, or candidiasis) and patients suffering from burning mouth sensation.
Al-Maskari AY, et al.[31]	2011	RA	Taste disorder	N/A	Oral manifestations and complications in patients with diabetes mellitus have been recognized and reported recently as a major complication of diabetes mellitus.
Ship JA [32]	2003	RA	Taste disorder	N/A	The goal of therapy is to promote oral health in patients with diabetes, to help prevent and diagnose diabetes in dental patients receiving routine stomatological care, and to enhance the quality of life for patients with this incurable disease.
Otero Rey EM, et al. [33]	2018	SR-MTA	Lichen planus	22 -S	In this study, the prevalence of planus was found in patients with diabetes, which ranged from 0.5% to 6.1%. % with a relative risk of 1.4
Shen ZY, et al. [34]	2012 [35]	RS	Lichen planus	518	The incidence of the history of systemic diseases, including DM, was not higher than expected when compared with the incidence reported in the general population.
Mozaffari HR, et al. [35]	2016	MTA	Lichen planus	11	The meta-analysis showed the risk of OLP in DM was higher compared with control subjects.
Kaomongkolgit R [36]	2010	CR	Lichen planus	1	The dental materials of the denture base play a fundamental role in the occurrence of OLP in the oral mucosa, especially the overextended denture flange that can induce a lichenoid reaction.

Table 1 (continued)

Summary of the reviewed articles.

Author(s)	Year of publication	Type of study	Pathology	No. of cases	Conclusion
Mneizel T. [38]	2005	IVS	Changes in the degree of keratinization	25	The wearing of complete acrylic dentures disturbs the denture-supporting epithelium of the palatal mucosa and results in decreased levels of epithelial keratinization.
Radke U, et al. [40]	2014	IVS	Changes in the degree of keratinization	30	Stimulation of the denture-bearing mucosa with astringent gum massage resulted in the gradual increase in keratinization.
Lindholm K, et al.[46]	1982	CCS	Changes in the degree of keratinization	67	Palatal keratinization under dentures was lower than the keratinization in the control group.
Farhan RS and as LS. [47]	2018	CCS	Changes in the degree of keratinization	75	Diabetes produces definite cytomorphometric changes in the oral mucosal cells of patients. The results suggested that nuclear diameter increased while cytoplasmic diameter decreased in T2M patients. The most predominant oral manifestations found in T2DM patients were periodontal disease and oral dryness.

CCS - *Case-Control Study*, CSS - *Cross-sectional study*, QS - *Quantitative study, RA - Review Article,* CS - *Cohort Study, POS - Prospective observational study*, *SR–MTA - Systematic review and meta-analysis, RS - Retrospectively Study*, *IVS - In Vivo Study, N/A – not applicable.*

A meta-analysis of 10 studies and 2 studies [21,23] with 107 DM patients showed a high incidence of candidiasis. One review article showed that the incidence of oral candidiasis in denture wearers has significantly increased. [20]

In a study by de Lima et al.,[24] which included 60 patients with oral lesions, there was no association between these lesions and the presence or absence of DM.Two studies with 362 patients showed that denture wearing in patients with DM was associated with an increased incidence of denture stomatitis (DS). In a study by Trentin et al.,[25] compared to nondiabetics, the stomatological manifestations were observed more frequently in T2DM patients and included pseudomembranous candidiasis, lichen planus, lingual varices, xerostomia, and prosthetic stomatitis. Among 129 patients with a mean age of 59.4 years, denture wear was associated with BMS in 58% of cases in a study by Mukatash-Nimri.[30]

In a review by Al-Maskari et al.,[31] among the many factors that influence changes in taste sensations in the oral cavity of DM patients, attention was also paid to the dysfunction of saliva, which contributes to changes in taste sensations.

A systematic review and meta-analysis performed by Otero Rey et al.[33] included 22 studies answering the following questions: «What is the prevalence and risk of OLP among patients with DM?» and «What is the prevalence and risk of DM among patients with OLP?» Twelve studies assessed the prevalence of DM among patients with OLP and reported ranges from 1.6% to 37.7% with a relative risk of 2.432. Ten studies assessed the prevalence of OLP among patients with DM and showed a prevalence ranging from 0.5% to 6.1% with a relative risk of 1.4.

A group of authors showed a decrease in the level of epithelial keratinization among those wearing complete acrylic dentures.[38,40,46] Farhan and Yas[47] conducted cytomorphometric measurements of oral mucosal cells in T2DM patients and healthy control subjects using exfoliated cytology smears. An eyepiece micrometer was used to take mean values of nuclear diameter (ND), cytoplasmic diameter (CyD), and the ratio of 2 diameters (N/C). The results showed a statistically significant increase in ND for buccal mucosa and the tongue, while CyD was decreased in T2DM patients; as a result, the N/C ratio for both the tongue and buccal mucosa significantly increased in T2DM patients, compared to controls.

Discussion

Given all the previously aforementioned oral manifestations in DM, the wearing of complete dentures in these patients is complicated by the higher incidence and gravity of oral mucosal lesions, compared to nondiabetic patients. Many studies describe the chronic complications of DM due to various metabolic and hemodynamic disturbances that mainly target vascular endothelial cells. The oral cavity is especially prone to complications.

One of the oral manifestations of patients with diabetes is xerostomia. Xerostomia is the subjective feeling of dry mouth, which is, in fact, a symptom, not a disease.[14] It is well known that a significant reduction of salivary flow leading to xerostomia is the most common oral manifestation of diabetes.[15] Ana Cicmil et al.[17] found a high presence of xerostomia in DM patients, compared to nondiabetics; these changes were especially pronounced in patients with poor glycemic control.According to Al-Dwairi et al.,[18] xerostomia adversely affects oral functions and overall satisfaction with dentures.

In wearers of a complete denture, the wetting mechanics of saliva are necessary to assist in the retention of prostheses. It has been reported that complete denture patients with xerostomia have more intense sore spots than patients with normal salivary flow. Also, OM becomes dry and tends to crack and ulcerate, which makes wearing removable prostheses uncomfortable.[19]

Diabetics have an increased predisposition to the manifestations of oral diseases like candidiasis, which

is associated with poor glycemic control and therapeutic dentures.[20] Wearing a complete denture is also known as an additional risk factor, which can promote colonization of *Candida*, produce *Candidal* biofilm, and result in oral candidiasis.[21]

Acrylic dentures are an important predisposing factor for oral candidiasis as these appliances, usually ill-fitting with suboptimal hygiene, act as reservoirs of infection.For instance, high salivary yeast counts are much more common in complete denture wearers than in dentate individuals.[22] In a study by Ganathy et al.,[23]15 subjects with complete acrylic prosthesis and DM were analyzed. The sample collection was carried out before and after oral hypoglycemicic drug intervention by swabbing the rugal surfaces of the palatal mucosa, followed by culture. A positive correlation was observed between oral candidiasis in the mucosa of complete denture wearers and elevated blood glucose levels. Oral hypoglycemic drug therapy positively controlled oral colonization by *Candida* in complete denture wearers with T2DM.In contrast, a study by de Lima et al. did not find significant differences between diabetic and nondiabetic subjects wearing complete dentures concerning salivary flow, salivary buffering capacity, denture retention, and oral mucosal lesions.

In a study by Trentin et al.,[25] diabetic patients with complete upper dentures had a higher incidence of DS than nondiabetics. This pathology is characterized by an erythematous lesion confined to the prosthesis area and may present with painful symptomatology. According to this study, T2DM metabolic control and oral lesions may have a significant relationship, as diabetic patients with complete upper dentures showed a 7.4 times higher risk than nondiabetics for developing DS.

A study by Khatibi et al.[26] showed a statistically significant association between T2DM and DS. A higher frequency of DS in DM patients indicates that DM causes alterations in the function of the host immune cells like polymorphonuclear leukocytes, monocytes, and macrophages.

BMS, presenting as burning pain, is a chronic, debilitating oral pain disorder characterized by generalized burning sensations on the tongue and other OM, with no discernible medical or dental causes.[27] Most individuals describe this symptom as a burning, tingling, scalding, annoying, tender, or numb feeling of the OM, most commonly involving the anterior two-thirds of the tongue, dorsum and lateral borders of the tongue, anterior portion of the hard palate, and labial mucosa.[28]

DM is one of the systemic conditions associated with secondary BMS. Burning mouth sensation in diabetic patients has been attributed to poor glycemic control, metabolic alterations in the OM, angiopathy, and neuropathy.[29]

Many factors have been implicated in altered taste sensation in the oral cavity.Taste dysfunction has been reported to occur more frequently in patients with poorly controlled DM, compared to healthy controls. Diabetic patients who suffer from neuropathy have a higher taste sensitivity threshold. Taste disturbance has also been reported to lead to poor glycemic control by inhibiting the ability to maintain a good diet.

OLP is a mucocutaneous inflammatory chronic disease with an overall prevalence of 1.27%.[33] Clinically, OLP may occur in 6 clinical variants: reticular, papular, plaque-like, erosive, atrophic, and bullous.[34] Because of the varied clinical forms of OLP, it is associated with various other systemic conditions, including DM. This association may be due to the endocrine dysfunction in DM and immunological defects.[35] Certain antidiabetic drugs in DM patients can cause an allergic reaction with a lichenoid-resulting response.[36] Also, denture-based dental materials play a fundamental role in the appearance of OLP in the OM. In the study by Rath et al.,[37] related to the impact of total prostheses on OLP, it was observed that the denture flange at the maxillary labial vestibule was a bit overextended. This might have caused repeated soft tissue impingement, causing a frank lesion with an exuberant lichenoid response.

Besides the previously mentioned oral manifestations in DM, several researchers have inquired about cellular changes in the OM of these patients. Cytology examination of the epithelial cells immediately under the acrylate prosthesis is an optimal non-invasive method. By exfoliative cytology, the epithelial cells are shed from the mucosal surface and applied to the glass slide. An appropriate cytobrush best obtains these. Some of these studies show that the oral epithelium under the prosthesis becomes more keratinized, while others show that the epithelium remains non-keratinized.[38] Some of the studies found that there is not only a quantitative reduction of keratinization but also acanthosis.[39-45] Keratinization, also termed cornification, is a process of cytodifferentiation during which the keratinocytes undergo maturation from their post-germinative state (basal layer) to finally differentiated, hardened cell filled with keratin, namely, stratum corneum. [39-45]

In a study by Lindfolm et al.,[46] the cytological effects of denture wear on the underlying palatal mucosa were analyzed in 67 complete denture patients and 44 persons without dentures (controls). Palatal keratinization under dentures was lower than the keratinization in the control group. Continuous denture wear, compared to daytime wear only did not reduce palatal keratinization. Mneizel,[38] using exfoliative cytology, showed that wearing complete maxillary acrylic dentures disturbs the denture-supporting epithelium of the palatal mucosa and decreases keratinization levels.

Conclusion

Based on the reviewed literature, the oral mucosal lesions in total acrylate prosthesis wearers may be summarized as follows: xerostomia, candidiasis, DS, oral mucosal lesions, BMS, taste disorders, OLP, and changes in degree of keratinization. There is a general agreement in the analyzed studies that in total acrylate prosthesis wearers who have DM, the gravity of the lesions is significantly higher than in nondiabetics. In DM patients, the keratinization of the oral mucosal cells is delayed. Adequate hygiene level is a contributing factor for fewer complications.

Competing Interests

The authors declare that they have no competing interests.

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International Journal of Biomedicine 13(4) (2023) 228-235
http://dx.doi.org/10.21103/Article13(4)_RA5

REVIEW ARTICLE

INTERNATIONAL JOURNAL OF BIOMEDICINE

Application of Non-Invasive Methods in the Treatment of White Spot Lesions in Children: A Review Article

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Abstract

This comprehensive review of 25 studies on white spot lesion (WSL) treatment in children highlights the effectiveness of resin infiltration (RI) and the potential of casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) as prominent treatment options. These findings are underscored by comparative studies, emphasizing the importance of long-term effects and age-specific considerations in treatment planning. The diversity in study types and diagnostic methods underscores the need for personalized approaches to WSL management. While common conclusions emerge, such as the efficacy of CPP-ACP and RI, contrasting findings remind us of the complexity of WSL management. The call for further research, particularly long-term studies, indicates the evolving nature of this field and the need for refining treatment protocols. Moreover, these studies emphasize the significance of conservative approaches, including regular oral hygiene practices and minimally invasive interventions, as integral components of a holistic WSL treatment strategy.**(International Journal of Biomedicine. 2023;13(4):228-235.)**

Keywords: white spot lesion • resin infiltration • casein phosphopeptide-amorphous calcium phosphate • fluoride-based treatment

For citation: Krasniqi B, Pavlevska M, Kurshumliu ED, Xhemaili BL, Kutllovc T. Application of Non-Invasive Methods in the Treatment of White Spot Lesions in Children: A Review Paper. International Journal of Biomedicine. 2023;13(4):228-235. doi:10.21103/Article13(4)_RA5

Abbreviations

CPP-ACP, casein phosphopeptide-amorphous calcium phosphate; **ICDAS II**, International Caries Detection and Assessment System II; **MID**, minimal intervention dentistry; **RI**, resin infiltration; **WSL**, white spot lesion.

Introduction

Dental caries, often known as tooth decay, is one of the most common chronic disorders afflicting people worldwide; people are susceptible to it throughout their lives.[1] The start and progression of caries are determined by the balance of pathogenic and preventative factors.[2] Dental caries is one of the most common diseases (approximately 50%) in children worldwide. If not treated in time, it can damage not only the mastication function, but also the child's speech, smile, and psychosocial environment, as well as the child's and family's quality of life. Dental disease treatment is quite expensive in all nations, and prevention is very easy and efficient.[3]

In November 2022, WHO reported 2.5 billion suffer from untreated caries.[4] Several studies have reported the

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prevalence of white spot lesions (WSLs) ranging from 2% to 97%.[5] In 1908, Black identified WSLs for the first time.[6,7] WSLs are described as demineralization of the enamel surface and subsurface without cavitation. These symptoms are the early clinical signs of dental caries development, with the possibility of reversal. These lesions are distinguished by their white, chalky, opaque appearance.[8]

At first, the mineral loss causes microporosities, which may be seen clinically as white, opaque, and rough areas. If the mineral loss persists, these WSLs will ultimately grow into lesions with cavitation.[9-11]

WSLs are common in orthodontic patients, especially around bracket bases and in areas where brushing is difficult.[12,13] Pathogenic bacteria enter the surface of the enamel and produce organic acids capable of dissolving the calcium and phosphate ions of the tooth structure, resulting in white spots.[8] One of the most prevalent side effects of fixed orthodontic treatment is early enamel decalcification, which presents as WSLs. Initially, a WSL has an intact top layer, followed by the more porous lesion body, which gives the lesion a chalky opaque look since light is dispersed primarily within the lesion body.[14] WSLs are frequently seen on the buccal surfaces of teeth, especially around brackets and in the gingival area.[14,15]

The WSL pathogenesis may be various. The main cause is related to plaque accumulation over time; moreover, many other factors, such as diet and levels of calcium, phosphate, bicarbonate, fluoride in saliva, and genetic factors, are reported.[16]

The primary objective of this review is to comprehensively explore and evaluate the utilization of non-invasive methods for the treatment of WSLs in children. In doing so, we aim to highlight the significance of non-invasive approaches within pediatric dentistry and emphasize their potential benefits.

Non-invasive methods in pediatric dentistry have garnered increasing attention due to their capacity to address WSLs without resorting to surgical interventions. Such approaches include remineralization therapies, fluoride treatments, and minimally invasive restorative techniques.[16] These methods aim to arrest the progression of WSLs and provide an opportunity for enamel remineralization, potentially reversing the damage and restoring the affected teeth to a healthy state.[17]

Resin infiltration (RI) therapy emerges as a prominent non-invasive option. This approach involves applying resin materials to demineralized enamel, effectively sealing and remineralizing the lesion without the need for drilling.[8,14] Another promising non-invasive approach is casein phosphopeptide-amorphous calcium phosphate (CPP-ACP), a remineralization agent. It can be used alone or with fluoride treatments to enhance enamel demineralization. Studies by Ebrahim et al. explored alternatives to traditional fluoride treatments, such as MI Paste Plus, to promote remineralization and reduce the progression of WSLs.[18] Minimal intervention dentistry (MID) principles emphasize conservative strategies that safeguard healthy tooth structure. Treatments like RI and CPP-ACP align with these principles.[19]

Although not a direct treatment, maintaining proper oral hygiene practices, including regular brushing and professional tooth cleaning, is crucial in managing WSLs and preventing their advancement.[17]

The significance of non-invasive methodologies in pediatric dentistry stems from their capacity to offer efficacious, streamlined, and less intrusive resolutions to a widespread dental issue among youngsters.[20] In contrast to invasive procedures that entail drilling and the substantial loss of tooth structure, non-invasive approaches prioritize the conservation of healthy tooth structure. This is particularly crucial when considering the dental health of youngsters, as their teeth are still growing and developing.

In addition, non-invasive techniques can provide a more pleasant and less anxiety-provoking encounter for pediatric patients,[20] which can positively influence their attitude toward dental care, decreasing fear and anxiety commonly associated with dental appointments and ultimately fostering improved adherence to preventative and therapeutic interventions.

Furthermore, non-invasive methodologies tend to be more cost-effective, benefiting both patients and healthcare systems.[19] Using conventional invasive treatment modalities, such as drilling and filling, presents several inherent difficulties. Frequently, these treatments require extracting sound dental tissue, resulting in potential tooth fragility and jeopardizing the tooth's sustained functionality.[20]

In light of the constraints associated with invasive interventions, there is an increasing demand for conservative options to manage WSLs successfully. Preserving good tooth structure is a primary concern in conservative treatments, as they aim to minimize the potential for iatrogenic harm and enhance patient experience.

Methods

The foundation of this study rested upon extensive electronic literature and secondary research conducted through renowned academic databases, including PubMed, MEDLINE, and Scopus. The study aimed to identify scholarly publications, clinical studies, case reports, original papers and review articles, predominantly in the English language, that pertained to the multifaceted domain of WSLs in pediatric dentistry. The choice of these databases was guided by their established reputation for housing an extensive repository of peer-reviewed articles, ensuring access to a comprehensive and relevant body of literature. We meticulously extracted data from the selected articles, from which relevant information regarding the study design, patient demographics, treatment methodologies, and outcomes was compiled. A structured framework facilitated the systematic organization of data, thus ensuring the synthesis of comprehensive and coherent insights.

Results and Discussion

We have summarized key findings from 25 scholarly articles focusing on WSL treatment in children (Table 1).

Table 1.
Summarized studies in a tabular format.

Authors	Type of Study	Number of Participants/ Objects	Treatment	Evaluation/ Diagnostic methods	Conclusion
Asokan et al. [21]	SR	Searched seven electronic databases, retrieved 158 clinical trials, assessed full text, included nine trials on CPP/ ACP	CPP-ACP	Visual scoring (ICDASII)	High-quality randomized controlled trials with low bias risk are required. CPP ACP showed a significant reduction in WSL size. Lack of evidence supporting other non-fluoridated agents.
Kannan & Padmanabhan [30]	RCT	12 patients with 240 WSLs in 193 teeth	Icon® RI and Clinpro™ XT varnish	Spectrophotometer for color assessment, DIAGNOdent® for fluorescence loss assessment	Immediately after the intervention, Icon® resin infiltration showed better color improvement. At 3 and 6 months, Clinpro™ XT varnish was more effective. Fluorescence loss improved with both interventions between immediate application and 6 months. At 6 months, Icon® resin-infiltrated WSLs were comparable to adjacent sound enamel, while Clinpro™ XT varnish-treated WSLs showed a significant difference.
Dhamija et al. [24]	RCT	60 patients	RI (Group 1) and Fluoride CPP-ACP varnish (Group 2)	ICDAS II scoring system, clinical examination	Resin infiltration was more successful than Fluoride CPP-ACP varnish in active non-cavitated WSLs.
Ebrahimi et al. [29]	RCT	80 patients	MI Paste Plus, Remin Pro, 2% Sodium Fluoride Gel, Usual Home Care (Control)	Intraoral photography, VistaCam iX for mineral content	MI Paste Plus and Remin Pro were as effective as 2% Sodium Fluoride in reducing WSLs in children. MI Paste Plus and Remin Pro are recommended as alternatives for managing WSLs.
Bourouni et al. [25]	SR/MA	11 studies, 1834 teeth in 413	RI	Visual-tactile, DIAGNOdent measurements	Resin infiltration has a significantly higher masking effect than natural remineralization or regular application of fluoride varnishes. However, this conclusion is based on only very few well-conducted RCTs. Resin infiltration seems a viable option to mask enamel white spot lesions and fluorosis esthetically.
Lopes et al. [32]	SR	143 references potentially relevant, with 99 publications from PubMed/Medline database, 33 from Scopus, and 11 from manual search.	SAPP11, CPP-ACP, HCL RI and fluoride-based products and chlorhexidine.	Visual examination or fluorescence,clinical photographs, cross-sectional microradiography, computed microtomography, DIAGNOdent.	There are no differences in the therapeutic approach for the treatment of white spot lesions, regardless of the type of diagnosis used.
Alexandru et al. [40]	R	N/A	RI technique (Icon by DMG) of the White Spot Lesion (WSL)	DIAGNOcam system, clinical evaluation, visual examination	An efficient and noninvasive diagnostic and monitoring method of the WSLs associated with infiltration therapy seems to be the ideal combination that follows the MID principles.
Güçlü et al. [33]	ClS	21 children with 101 WSLs	Four treatment groups: FV, CPP-ACP, CPP-ACP-FV, Control	Visual appraisals and laser fluorescence (LF) measurements.	Self-applications of CPP-ACP paste significantly improved the appearance and remineralization of WSLs. No advantage was observed for the use of fluoride varnish as a supplement to either the standard or CPP-ACP-enhanced oral hygiene regimes.
Bergstrand & Twetman [29]	R	25 publications that fulfilled human clinical trial criteria	Topical fluorides, fluoride toothpaste, professional applications of FV, remineralizing cream sugar alcohols, probiotics	Literature review, clinical trials with surrogate endpoints	The review highlights the importance of topical fluoride varnish and other interventions in preventing and treating WSLs during and after orthodontic treatment. Further research is needed to strengthen these findings.
Pintanon et al. [42]	IVtS	N/A	Caries infiltration (Icon), CPP-ACP containing paste (Tooth Mousse)	Vickers diamond indenter for surface hardness, spectrophotometer for color change, SEM for microscopic observation	Caries infiltration with resin material immediately improved surface hardness and esthetic appearance of artificial white spot lesions. CPP-ACP paste did not.

Table 1 (Continued).
Summarized studies in a tabular format.

Authors	Type of Study	Number of Participants/ Objects	Treatment	Evaluation/ Diagnostic methods	Conclusion
Ciftci et al. [34]	OA	68 patients (41 females and 27 males), 137 teeth	RI for developmental enamel opacities and WSLs, FV for WSLs	ICDAS II criteria, DIAGNOdent Pen scores	Resin infiltration was more successful than fluoride varnish for WSLs. Continuous fluoride application is needed for a similar effect. Resin infiltrate partially masked opaque lesions in developmental enamel opacities.
Simon et al. [22]	RCCT	60 children	RI (ICON, DMG, Germany) and Casein Phospho Peptide (CPP-ACP, Toothmousse, GC corporation, Germany)	Identification of WSLs with ICDAS I criteria, size measurement using clinical photographs, color assessment with a spectrophotometer, area calculation with Digimizer software	Both resin infiltration and CPP-ACP showed a significant reduction in lesion area and improved color of white spot lesions. They are recommended for managing post-orthodontic white spot lesions when the proper protocol is maintained.
Giray et al. [26]	RCT	23 children	RI vs. FV	Laser fluorescence device (DIAGNOdent pen, Kavo, Germany).	Resin infiltration and fluoride varnish are clinically feasible and efficacious methods for the treatment of anterior WSLs. The inhibition of caries progression by resin infiltration should now be considered an alternative to topical fluoride treatment.
Abbas et al. [14]	IVS	96 participants	RI with variations in etching and infiltrant applications	Spectrophotometric analysis of color change	Shallow enamel lesions were best treated with one etching and one infiltrant application. Deep enamel and shallow dentine lesions were best treated with one etching and two infiltrant applications. Deep dentine lesions required one etching with two infiltration steps but remained clinically detectable. As the WSLs got deeper, they became more clinically visible.
Alamoudi et al [35]	CR	Two patients	RI treatment for non-cavitated WSLs on anterior maxillary teeth	Patient interviews immediately after treatment and two weeks post-treatment, and examined with ICADS	Resin infiltration is a comfortable, conservative treatment option providing satisfactory outcomes in masking non-cavitated white spot lesions on anterior maxillary teeth after orthodontic treatment.
Ferreira et al. [36]	RCT	15 children	Two varnish formulations (G1 = 5% NaF, G2 =6% NaF + 6% CaF2) for controlling white spot lesions	Dimensional measurements of WSL, Clinical features in a visual manner (texture and brightness), Activity classification	After 4 applications, both varnish formulations produced similar clinical effects, reducing and controlling carious activity in most WSL.
Eckstein et al. [37]	IVtS	9 subjects (49 trial teeth)	RI with Icon, DMG, Hamburg, Germany	CIE-Lab color assessment.	Resin infiltration effectively improved the appearance of post-orthodontic WSLs and maintained these cosmetic improvements over a 12-month period. The color and lightness characteristics of the Icon infiltrant remained stable, supporting its use for the long-term esthetic improvement of post-orthodontic WSLs.
Doméjean et al. [39]	SR	14 Studies used in the review but only 4 met the inclusion criteria	RI	Review of 4 in vivo studies	RI appears effective in arresting non-cavitated caries lesions. Additional long-term studies are needed.
Paris et al. [38]	IVvS	120 bovine enamel samples	Application of experimental infiltrants and commercial infiltrant (Icon, DMG)	Photographic images, color differences (ΔE) compared with untreated enamel	Resin infiltration can mask artificial enamel caries lesions, reducing color differences. Refractive indices of infiltrants may influence the aesthetic outcome and susceptibility to staining. Further research is needed.

Table 1(Continued).
Summarized studies in a tabular format.

Authors	Type of Study	Number of Participants/ Objects	Treatment	Evaluation/ Diagnostic methods	Conclusion
Memarpour et al. [23]	RCT	140 children	4 groups: 1. Control (no intervention) 2. Oral hygiene and dietary counseling 3. Oral hygiene and fluoride varnish application 4. Oral hygiene and tooth mousse (CPP-ACP) application	Size of WSL in millimeters and dmft index.	Oral hygiene, along with four fluoride varnish applications or constant CPP-ACP during the 12-month period, reduced the size of WSL in the anterior primary teeth and caused a small increase in the dmft index values.
Baafif et al. [41]	CS	30 participants	Split-mouth technique used, treating WSLs on the left side with ICON and on the right side with CPP-ACFP	Clinical evaluation using DIAGNOdent.	Both ICON and CPP-ACFP were effective in treating WSLs of smooth surfaces, but the efficacy of CPP-ACFP was better than ICON.
Elrashid et al. [43]	SR&MA	From 106 studies identified, 17 assessed	RI of proximal carious lesions	Risk of bias assessment, meta-analyses using RevMan software	The available evidence conveys high confidence that proximal resin infiltration has superior efficacy in slowing/arresting the carious lesions' progression rate in comparison to conventional management modalities.
Mendes et al. [27]	RCT	36 individuals	CPP-ACP	Monitoring with DIAGNOdent Pen	The use of CPP-ACP is a good alternative for the remineralization of white-spot lesions. The effect can be improved when this product is applied in combination with fluoride.
Gözetici, et al [31]	CS	134 participants	RI, P11-4, FV, Control	LAA-ICDAS scores, LF Pen measurements	Lesion regression was observed in all groups after six months, encouraging non-operative treatment approaches. Regular brushing and professional tooth cleaning are effective. Resin infiltration or fluoride varnish may enhance lesion improvement in moderate- to high-caries-risk individuals.

SR *- Systematic Review,* ***RCT*** *- Randomized Clinical Trial,* ***SR/MA*** *- Systematic Review/Meta-analysis,* ***ClS*** *- Clinical Study,* ***R*** *- Review,* ***IVtS*** *- In Vitro Study,* ***OA****- Original Article,* ***RCCT*** *- Randomized Control Clinical Trial,* ***CR*** *- Case Report,* ***IVvS*** *– In Vivo Study,* ***SR&MA*** *- Systematic Review & Meta-Analysis,* ***CS*** *- Comparative Study,* ***Icon®****- A brand or product name for resin infiltration,* ***Clinpro™ XT*** *- A brand or product name for varnish,* ***MI Paste Plus*** *- A product name,* ***SAPP11*** *- Self-Assembling Peptide,* ***HCL*** *- Hydrochloric Acid,* ***FV*** *- Fluoride Varnish,* ***GC Corporation*** *- A company or brand name,* ***DMG*** *- A company or brand name,* ***NaF*** *- Sodium Fluoride,* ***CaF2*** *- Calcium Fluoride,* ***P11-4*** *- A specific peptide or compound,* ***LF****- laser fluorescence,* ***DIAGNOdent*** *- scans a clean tooth surface with a laser beam,* ***ICDAS-LAA*** *- International Caries Detection and Assessment System—Lesion Activity Assessment;*

These studies are presented in a tabular format, simplifying the presentation and allowing direct comparisons between them. The analysis of these studies reveals a rich landscape of research into diverse treatment modalities, diagnostic methods, and patient populations. RI stands out as an effective and minimally invasive treatment option, while CPP-ACP shows promise as a remineralization agent. Comparative studies provide valuable insights, and long-term effects and age-specific considerations are essential for treatment planning. As the research on WSL treatment advances, it is clear that personalized, conservative approaches are gaining prominence. The call for further research underscores the dynamic nature of the field, and ongoing studies will likely refine and expand our understanding of WSL management. Ultimately, the goal is to provide effective, evidence-based treatments that improve oral health outcomes and the quality of life for individuals, especially children, affected by WSLs.

One striking feature of the analyzed studies is the diversity in study types, ranging from systematic reviews and randomized controlled trials to clinical studies, in vitro experiments, and case reports. This diversity reflects the multifaceted nature of WSLs and underscores the importance of investigating them through various research lenses.

Numerous authors exploring the intricacies of WSL management share striking similarities in their research approaches. This convergence of methodologies and findings underscores the significance of their collective contributions to this field of study.

Some common conclusions were found regarding CPP-ACP for WSL size reduction. Asokan et al. conducted

a systematic review emphasizing the significant reduction in WSL size with CPP-ACP treatment.[21] This theme resonates across multiple studies, including Simon et al.[22] and Memarpour et al.[23] endorsing CPP-ACP efficacy in addressing these lesions. As for the efficacy of RI, its effectiveness in managing WSLs is a consistent finding across various studies. Dhamija et al.,[24] Bourouni et al.,[25] and Giray et al.[26] affirm the superiority of RI over other treatments, such as fluoride CPP-ACP varnish or fluoride varnish. RI for post-orthodontic WSLs was a topic discussed by Simon et al. and Giray et al., which specifically highlighted the effectiveness of RI in managing post-orthodontic WSLs, recommending its use when proper protocols are maintained.[22,26]

As for the fluoride alternatives, studies such as Memarpour et al.,[23] Mendes et al.,[27] and Ebrahimi et al.[28] underscore the viability of alternatives to fluoride, such as MI Paste Plus or CPP-ACP, for managing WSLs.

According to a study conducted by Bergstrand and Twetman,(29) the literature they reviewed underscores the significance of utilizing topical fluoride varnish and other interventions in preventing and treating WSLs during and after orthodontic treatment. However, the study also highlights the need for further research to confirm and strengthen these findings.

The authors concluded that the use of remineralizing agents can be beneficial in the management of WSLs in children.[23,28,29]

Throughout the literature, we also witnessed contrasting conclusions. The idea that color would be improved over time was discussed by Kannan and Padmanabhan, who found that Icon® RI showed immediate color improvement, while Clinpro™ XT varnish was more effective at 3 and 6 months.[30] Conversely, Dhamija et al.[24] favored RI over fluoride CPP-ACP varnish, indicating differing outcomes in terms of color improvement over time.

We also found a debate about RI vs. fluoride varnish where Gözetici et al.[31] compared RI to fluoride varnish and observed lesion regression in all groups after six months. In contrast, Giray et al. supported RI as a viable alternative to fluoride varnish but noted that continuous fluoride application might be necessary for similar effects.[26]

The studies employed various diagnostic methods, including visual scoring (ICDASII), spectrophotometry, fluorescence assessments (DIAGNOdent), and more. Authors such as Kannan & Padmanabhan,[30] Soveral et al.,[6] and Lopes et al.[32] employed advanced diagnostic tools, like spectrophotometers and DIAGNOdent, to assess color and mineral content. On the other hand, studies like Güçlü et al.[33] and Memarpour et al.[23] incorporated visual appraisals and laser fluorescence measurements. These varying diagnostic methods underline the diversity of approaches in assessing WSLs.

There were also similarities in diagnosis, treatment, and conclusions. Simon et al.[22] and Giray et al.[26] explored RI as a treatment option. They also shared similarities in diagnostic methods, using visual scoring and spectrophotometry. While not identical, their conclusions highlighted the efficacy of RI in managing WSLs.

These studies collectively contribute to our understanding of WSL management, with recurring themes such as the efficacy of CPP-ACP and RI. However, differences in treatment outcomes and diagnostic approaches remind us of the complexity of these lesions and the need for ongoing research to refine treatment protocols further.

While some studies found certain treatments more effective than others, there is a consensus that non-invasive approaches can successfully manage WSLs, especially when combined with proper oral hygiene practices. Among the numerous treatment modalities explored, RI emerges as a prominent and effective option.[14-16,22,24-26,34-37] RI therapy showed significant promise in reducing the size of WSLs and enhancing esthetic outcomes.[38,39] The studies uniformly recommend it as a viable alternative to traditional fluoride treatments.[14-16,22,24-26,34-37] Its minimally invasive nature aligns with the MID principles, emphasizing conservative approaches that preserve tooth structure.

The studies show that the most commonly used treatment methods for WSLs are RI and fluoride-based treatments, particularly fluoride varnish and CPP-ACP.[14-16,22,24-26,34-37] These methods are frequently mentioned and evaluated across multiple studies. RI, in particular, stands out as a prominent treatment option.

As for the methods of diagnosis and evaluation, visual examination and scoring systems, such as the ICDASII, are commonly used. Additionally, some studies incorporate diagnostic tools like DIAGNOdent to assess fluorescence loss and spectrophotometers to assess color.[24,34] The choice of diagnostic methods varies across the studies, including visual scoring based on ICDASII criteria, spectrophotometry, DIAGNOdent assessments, clinical examinations, and more. This variance in diagnostic tools underscores the need for a personalized approach to WSL management, allowing clinicians to tailor their interventions based on specific patient needs and lesion characteristics.[40]

RI and CPP-ACP are two standout treatments in the quest to manage WSLs effectively.[23,41] RI demonstrated significant success in both arresting lesion progression and improving esthetics. Multiple studies advocate for its clinical feasibility and consider it a valuable alternative to topical fluoride treatment.[14-16,22,24-26,34-37] On the other hand, CPP-ACP, a non-invasive remineralization agent, displayed potential when used alone or in combination with fluoride.[22,28,32,42] Its capacity to enhance remineralization is encouraging, particularly for managing non-cavitated WSLs.

Comparative studies play a crucial role in assessing the relative efficacy of different treatment options. These studies provide valuable insights into which interventions are more effective in specific scenarios. For instance, the comparison between RI and fluoride varnish indicated that both are clinically feasible and productive methods, though RI offers a notable alternative to traditional fluoride approaches.[14-16,22,24-26,34-37] Several studies explored the long-term effects of treatments, highlighting the significance of follow-up and monitoring. RI, for instance, maintained cosmetic improvements over an extended period. Moreover, the age of the patient population was a variable in these studies, emphasizing the need for age-

specific treatment strategies and highlighting the challenges and opportunities presented by different age groups.

Conclusion

In conclusion, the extensive analysis of 25 studies on WSL treatment in children reveals a diverse landscape of research, showcasing the effectiveness of RI and the potential of CPP-ACP as prominent treatment modalities. Comparative studies provide valuable insights, emphasizing the importance of long-term effects and age-specific considerations in treatment planning. The diversity in study types and diagnostic methods highlights the need for personalized approaches to WSL management. While common conclusions emerge, such as the efficacy of CPP-ACP and RI, contrasting findings underscore the complexity of WSL management. A recurring theme in these studies is the call for additional research, especially long-term studies, to strengthen the evidence base for WSL treatment options. This indicates that the field is continually evolving, and there is room for further refinement of treatment protocols. Furthermore, many studies underscore the importance of conservative approaches to WSL management, emphasizing regular brushing, professional tooth cleaning, and minimally invasive interventions as key components of a holistic treatment strategy.

Competing Interests

The authors declare that they have no competing interests.

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International Journal of Biomedicine 13(4) (2023) 236-239
http://dx.doi.org/10.21103/Article13(4)_BR

BRIEF REVIEW

INTERNATIONAL JOURNAL OF BIOMEDICINE

The Role of Topical Finasteride in Hair Loss Management: Current Evidence and Future Perspectives

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Abstract

Hair loss (HL), scientifically known as alopecia, is a prevalent condition affecting individuals globally. Its multifactorial origins encompass genetics, hormonal fluctuations, stress, aging, medical conditions, and medications. The psychological impact of HL on self-esteem, confidence, and overall quality of life has driven the pursuit of effective treatments to rejuvenate hair growth and appearance. Among these treatments, finasteride (FIN) has been employed to address male pattern HL, the most prevalent form of HL in men. This medication hinders the conversion of testosterone to dihydrotestosterone (DHT), a hormone that contributes to hair follicle shrinkage and cessation of hair production. While oral FIN has been widely explored, it entails undesirable side effects and varying effectiveness. This review delves into the role of FIN topical applications as a novel approach for HL treatment.**(International Journal of Biomedicine. 2023;13(4):236-239.)**

Keywords: hair loss • hair follicle • finasteride • dihydrotestosterone

For citation: Hussein RS. The Role of Topical Finasteride in Hair Loss Management: Current Evidence and Future Perspectives. International Journal of Biomedicine. 2023;13(4):236-239. doi:10.21103/Article13(4)_BR

Introduction

Hair loss (HL) is a prevalent condition that impacts millions of individuals around the globe. It can have various causes, such as genetics, hormones, stress, aging, disease, or medication. HL can affect one's self-esteem, confidence, and quality of life. Therefore, many people seek effective treatments to restore their hair growth and appearance.[1]

One of the treatments used for male pattern HL, which is the most common type of HL in men, is finasteride (FIN). FIN is a medication that works by blocking the conversion of testosterone to dihydrotestosterone (DHT), which is the hormone that causes hair follicles to shrink and stop producing hair. FIN can be taken orally as a tablet or applied topically as a solution or gel.[2]

However, oral FIN may have undesirable side effects, such as decreased libido, erectile dysfunction, and gynecomastia. Although topical FIN may not be able to deliver enough concentration to the hair follicles to have a significant effect, some researchers have explored the possibility of using it as an alternative method of oral use in androgenetic alopecia.[3]

Topical FIN involves applying the drug to the scalp at the sites of HL. The aim is to deliver a higher concentration of FIN to the hair follicles and avoid systemic side effects. However, the role of FIN topical application in treating HL is not well established. There is limited evidence from clinical trials to support its efficacy and safety. Moreover, there are different protocols for the dose, frequency, and duration of FIN topical application, making it difficult to compare the results.[4]

This paper reviews the current literature on FIN topical application for HL and discusses its potential benefits and drawbacks. We will also suggest some directions for future research and clinical practice on this topic.

The Mechanism of HL and the Role of DHT

The hair growth cycle is a complex process consisting of distinct phases: anagen, catagen, and telogen. These phases collectively regulate the growth, rest, and shedding of hair. The anagen phase is an active growth phase, during which hair follicles produce and elongate hair strands. The duration of the anagen phase varies across individuals, contributing to

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differences in hair length. Healthy hair growth primarily occurs during this phase. Following the anagen phase, hair follicles enter the catagen phase, also known as the transitional phase. This short period involves the cessation of hair growth and the shrinking of the hair follicle. The hair shaft is disconnected from the blood supply during this stage. The telogen phase is the resting phase, during which hair follicles are relatively dormant. The old hair remains attached to the follicle but is eventually shed to make way for new hair during the next anagen phase.[5,6]

Various factors can disrupt the normal hair growth cycle, leading to HL. Genetic predisposition, hormonal imbalances, age, stress, and certain medical conditions can influence the duration of each phase and the overall balance of the cycle.[7] DHT plays a critical role in the progression of HL, particularly in androgenetic alopecia, commonly known as male pattern baldness. DHT is derived from testosterone through the action of the enzyme 5-alpha-reductase. This conversion occurs predominantly in hair follicles, skin, and the prostate.[8] While DHT is necessary during puberty to develop male secondary sexual characteristics, its excessive presence in hair follicles can lead to hair miniaturization. DHT binds to androgen receptors present on hair follicles, particularly those on the scalp. This binding triggers a process called miniaturization, where the affected hair follicles become progressively smaller with each growth cycle. As a result, the hairs produced become finer and shorter in length. Eventually, the hair follicles can become unable to produce visible hair strands, leading to baldness.[9]

Male pattern HL is characterized by a distinct pattern of hair thinning and balding. It typically begins with the recession of the hairline, followed by thinning of the crown area. DHT's influence on hair follicles leads to the gradual transformation of terminal hairs (thick, pigmented hairs) into vellus hairs (fine, colorless hairs), rendering the affected areas visibly sparse.[10]

Understanding the role of DHT in HL is pivotal for devising targeted interventions to counteract its effects. This knowledge has fueled the exploration of treatments aimed at inhibiting DHT's impact on hair follicles, including the use of FIN and the novel approach of FIN topical application.[11]

Current Treatment Approaches for HL

Oral FIN has been a prominent treatment option for addressing HL, mainly male pattern HL. FIN, administered orally, functions as a 5-alpha-reductase inhibitor, specifically targeting the Type II enzyme. By inhibiting the conversion of testosterone to DHT in the body, FIN aims to reduce the levels of DHT that contribute to hair follicle miniaturization.[12] Clinical studies have demonstrated the potential of oral FIN to slow down HL progression and promote regrowth in some individuals.[13] While oral FIN offers a systemic approach to mitigating DHT's effects, it is associated with potential adverse effects. These may include decreased libido, erectile dysfunction, gynecomastia, and mood alterations. Concerns about these side effects have led to hesitation among some patients, particularly those who prioritize their sexual and hormonal well-being.[3]

The topical application of FIN has emerged as an alternative to oral administration, aimed at targeting the scalp more directly to provide a localized treatment option with reduced systemic exposure. While its mechanism of action remains similar to oral FIN, topical application could potentially reduce the risk of systemic side effects.[14] Studies investigating the efficacy of topical FIN have shown promising results in hair growth and maintenance, although direct comparisons with oral FIN require further research. One of the challenges of topical FIN lies in achieving consistent and sufficient drug delivery to hair follicles. The scalp's barrier function and the need for optimal absorption can make it difficult to achieve the desired therapeutic effect. Ensuring a standardized and effective formulation for topical application poses a significant difficulty.[15,16]

Topical Finasteride: The Concept and Rationale

Topical FIN introduces a unique strategy for delivering the drug directly to the scalp's hair follicles. The topical method bypasses the barriers presented by oral ingestion. This targeted delivery[17]) aims to enhance treatment efficacy by addressing the root cause of HL at the local level. Unlike oral administration, which exposes the entire body to the drug, topical application minimizes systemic absorption. This targeted approach seeks to mitigate the risk of systemic side effects, as the FIN concentration remains primarily confined to the treated area.[18]

The concept of topical FIN holds theoretical advantages, along with potential drawbacks and uncertainties that warrant careful consideration. Direct topical application enhances the concentration of FIN near the hair follicles. This higher drug concentration theoretically promotes more effective inhibition of DHT at the site of action, potentially enhancing hair regrowth and maintenance.[19] By systemic circulation, topical FIN aims to minimize the likelihood of systemic adverse effects, such as decreased libido and erectile dysfunction. This could make the treatment more appealing to individuals concerned about the systemic impact of traditional administration methods.[20]

While the concept is intriguing, there remain uncertainties regarding the optimal topical technique, dosing regimen, and potential safety concerns. The procedure requires careful consideration to avoid complications and ensure patient comfort. The long-term safety profile and potential local adverse effects also require comprehensive evaluation. It becomes clear that while the concept holds promise, rigorous clinical investigations are essential to validate its efficacy, safety, and potential as an alternative treatment modality for HL.[21]

Existing Evidence: Clinical Studies and Findings

Published studies have employed various study designs, ranging from randomized controlled trials to case series and observational studies. These studies have investigated different aspects of topical FIN, including dosing regimens, application techniques, and treatment durations.[14] Research findings have provided insights into the potential efficacy of topical FIN in promoting hair regrowth and reducing HL. Some studies have reported positive outcomes, including increased hair density and improvements in hair follicle health. Safety assessments have explored local and systemic adverse effects, shedding light on the potential tolerability of this treatment modality.[18,20]

While the existing evidence sheds light on the potential of topical FIN, several discrepancies and limitations within the literature warrant consideration. There is a lack of standardized protocols regarding the dose, frequency, and duration of FIN topical application. Different studies have employed diverse regimens, making comparing results and establishing a consensus on the optimal approach challenging.[22] Many published studies suffer from small sample sizes and relatively short follow-up periods. This limits the generalizability of findings and the ability to assess the treatment's long-term efficacy and safety. Comprehensive, larger-scale studies with extended follow-up are needed to provide more robust evidence.[15]

The absence of standardized outcome measures complicates the interpretation of study results. Variation in assessment tools, such as hair density measurements, patient-reported outcomes, and clinical evaluations, hinders the ability to draw definitive conclusions about treatment efficacy. As the research landscape on topical FIN continues to evolve, these discrepancies and limitations underscore the need for well-designed studies that adhere to standardized protocols, utilize larger sample sizes, and employ consistent outcome measures.[23]

Future Directions for Research and Clinical Practice

The key areas where future investigations can contribute to refining treatment protocols and enhancing the understanding of the topical FIN innovative approach may include:

A. Standardization of Application Protocols

1. Establishing standardized guidelines for dosing, application frequency, and treatment duration; and identifying optimal regimens that balance therapeutic efficacy with safety will contribute to a more cohesive body of evidence.

2. Collaboration between researchers and clinicians is pivotal in developing comprehensive and consistent topical protocols. Sharing experiences and insights can lead to a consensus on best practices, ensuring that future studies build upon a foundation of standardized approaches.

B. Long-Term Efficacy and Safety Assessments

1. To address lingering uncertainties surrounding topical FIN's long-term outcomes and safety profile, researchers must conduct studies with extended follow-up periods. This will help capture the effect of treatment on durability and on potential late-emerging safety concerns.

2. Conducting larger-scale studies with more diverse participant groups and longer observation periods can provide a clearer understanding of the treatment's efficacy and safety. Long-term assessments are essential for evaluating the treatment's potential for sustained benefits.

C. Comparative Studies with Existing Treatments

1. Comparative studies pitting topical FIN against existing treatments, such as oral FIN, oral minoxidil and topical minoxidil, can offer insights into relative efficacy, safety, and patient preferences. These studies enable clinicians to make informed decisions when tailoring treatment plans to individual needs.

2. Investigating the synergistic effects of combining topical FIN with other interventions, such as minoxidil or low-level laser therapy, holds promise. Such combinations could potentially amplify treatment outcomes by targeting multiple pathways involved in hair growth and maintenance.

As research on topical FIN advances, adherence to these future directions will contribute to the accumulation of robust evidence and the refinement of clinical practice.[24]

Conclusion

The pursuit of effective HL treatments is ongoing, driven by the desire to enhance the quality of life for those affected. The use of FIN topically promises advancement in this endeavor, offering the potential to address HL at its source while minimizing systemic side effects. As research continues and the evidence base matures, the landscape of HL management is poised to benefit from new insights and approaches that can make a meaningful impact on the lives of individuals worldwide.

Competing Interests

The authors declare that they have no competing interests.

Acknowledgments

This study was supported by Prince Sattam bin Abdulaziz University (Project PSAU/2023/R/1444).

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International Journal of Biomedicine 13(4) (2023) 240-245
http://dx.doi.org/10.21103/Article13(4)_OA1

ORIGINAL ARTICLE

Cardiology

INTERNATIONAL JOURNAL OF BIOMEDICINE

Left Ventricular Function after Revascularization in Patients with Chronical Coronary Syndromes

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Abstract

The purpose of this study was to determine the dynamics of morpho-functional and myocardial deformation characteristics of the left ventricle after revascularization in patients with chronic coronary syndromes (CCS).

Methods and Results: The study included 136 CCS patients of both sexes with stable anginal symptoms [(i) clinical scenario] and asymptomatic coronary artery disease (CAD) at screening [(vi) clinical scenario]. Diagnosis of CCS was performed according to the 2019 ESC Guidelines for the diagnosis and management of chronic coronary syndromes. All patients underwent the following examinations: assessment of traditional risk factors, physical examination, general clinical and laboratory blood tests, 12-lead ECG, transthoracic echocardiography, two-dimensional speckle tracking echocardiography (STE), and coronary angiography (CAG). The SYNTAX score was calculated retrospectively according to the SYNTAX score algorithm. A total of 100 patients with CCS were enrolled in the main group (MG) and underwent revascularization by PCI with intracoronary stenting using drug-eluting stents. Among the main-group patients, one-vessel, two-vessel, and three-vessel CAD were detected in 36(26.5%), 34(25%), and 30(22.0%) cases, respectively. The comparison group (CG) included 36 CCS patients with hemodynamically non-significant coronary lesions.

LVEF values were within the normal range in all groups, with the highest value in the CG, followed by the one-, two- and three-vessel lesion groups. LVEF obtained by the area-length method and modified biplane Simpson's method did not differ. The assessment of the contractile function of the LV myocardium was also obtained by assessing the global longitudinal strain (GLS) and global longitudinal strain rate (GLSR). The comparative analysis of the LV myocardial deformation properties in the studied groups showed that less negative GLS and GLSR were found in the three-vessel CAD, followed by the two-vessel and one-vessel CAD groups, and CG. CG demonstrated more negative GLS and GLSR than all MG subgroups.

We found no statistically significant differences in the GLS before and 48 hours after revascularization in all studied MG subgroups and CG. Thirty days after revascularization, GLS significantly showed more negative values in all MG subgroups: -18.12±0.63 versus -17.9±0.4 in one-vessel CAD, -16.13±0.71 versus -15.9±0.4 in two-vessel CAD and -13.91±1.25 versus -13.1±1.1 in three-vessel CAD. In CG with medical treatment only, GLS did not change statistically significantly but had more negative values than in the studied MG subgroups. Analysis of changes in LVEF after revascularization in the MG of patients with one-, two- and three-vessel CAD and in the CG after medical treatment did not reveal statistically significant dynamics.

Conclusion: the results indicate the absence of statistically significant changes in myocardial deformation indicators and morpho-functional parameters of the left ventricle in CCS patients 48 hours after revascularization. Thirty days after revascularization, GLS significantly improves, while LVEF remains unchanged. GLS is superior to LVEF in visualizing improvement in LV function after revascularization in patients with CCS.**(International Journal of Biomedicine. 2023;13(4):240-245.)**

Keywords: coronary artery disease • speckle tracking echocardiography • global longitudinal strain

For citation: Doniyorov ShN, Bekmetova FM, Bekbulatova RSh, Fozilov KhG, Mukhamedova MG, Yuldashev NA, I'lkhomova LT, Arslonov SF, Karimov BS. Left Ventricular Function after Revascularization in Patients with Chronical Coronary Syndromes. International Journal of Biomedicine. 2023;13(4):240-245. doi:10.21103/Article13(4)_OA1

Abbreviations

2D-STE, 2-dimensional speckle tracking echocardiography; **ASE**, American Society of Echocardiography; **ACS**, acute coronary syndromes; **CAD**, coronary artery disease; **CAG**, coronary angiography; **CCS**, chronic coronary syndromes; **CABG**, coronary artery bypass grafting; **GLS**, global longitudinal strain; **GLSR**, global longitudinal strain rate; **LVEDV**, left ventricular end-diastolic volume; **LVESV**, left ventricular end-systolic volume; **LV**, left ventricle; **LVEF**, left ventricular ejection fraction; **MI**, myocardial infarction; **PCI**, percutaneous coronary intervention; **SR**, strain rate.

Introduction

Coronary artery disease (CAD), also called coronary heart disease or ischemic heart disease, is the most common cause of death in developing and developed countries. CAD, characterized by the accumulation of atherosclerotic plaque in the epicardial arteries, with or without obstruction, can have long stable periods but can also become unstable due to an acute atherothrombotic event caused by plaque rupture or erosion.[1] The chronic course of the disease is most often progressive, even in clinically silent periods. The dynamic course of CAD leads to clinical conditions that can be divided into acute coronary syndromes (ACS) and chronic coronary syndromes (CCS).

In 2019, the European Society of Cardiology presented "Guidelines for the diagnosis and management of chronic coronary syndromes."[1] The Guidelines describe 6 clinical scenarios for CCS that carry different risks for future cardiovascular events [e.g., death or myocardial infarction]. This risk may be increased by poor control of cardiovascular risk factors, suboptimal drug therapy, or failed revascularization.

In CCS patients, optimal medical therapy reduces clinical symptoms and the progression of atherosclerosis, and prevents atherothrombotic events. In addition to medical therapy, myocardial revascularization via percutaneous coronary intervention (PCI) or coronary artery bypass grafting (coronary artery bypass grafting) effectively reduces angina symptoms and antianginal drug use, and improves exercise capacity and quality of life, compared with medical treatment alone.

The 5-year follow-up of the FAME 2 (Fractional Flow Reserve versus Angiography for Multivessel Evaluation 2) trial showed that in patients with stable angina and angiographically significant stenoses, fractional flow reserve (FFR [<0.80])-guided PCI plus medical therapy was associated with a significantly lower rate of the primary composite end point of death, myocardial infarction (MI), or urgent revascularization than medical therapy alone.[2] However, the individual risk-benefit ratio should be assessed, and revascularization should only be considered if the expected benefit outweighs the potential risk.[1]

In CCS patients, left ventricular ejection fraction (LVEF), evaluating left ventricular systolic function, is often normal.[3] Myocardial deformation analysis evaluates LV mechanics by quantifying strain and strain rate (SR). Global longitudinal strain (GLS), assessed by 2D-STE, is a sensitive method for assessing LV function.[4] GLS expresses the longitudinal shortening as a percentage (change in length as a proportion to baseline length). Reference values for normal longitudinal strain from a recent meta-analysis using transthoracic echocardiography are 19.7±0.4% (mean±SD).[5] GLS may be altered despite preserved LV function, as assessed by LVEF, in conditions predisposing to cardiovascular disease, including older age, hypertension, diabetes mellitus, stable angina, renal dysfunction, and obesity.[6-11] In addition, GLS has prognostic value in patients with cardiovascular diseases, including acute MI[12] and heart failure with reduced[13,14] and preserved LVEF.[15] In the general population, GLS provides independent and complementary prognostic information regarding the long-term risk of cardiovascular morbidity and mortality.[16] The speed of myocardial deformation is also important and is characterized by SR.[17,18] Average longitudinal systolic SR in subjects without cardiovascular disease measured by transthoracic echocardiography is -1.10±0.16 sec^{-1}.[19] It should be noted that GLS correlates with LVEF, and SR correlates with the rate of rise of LV pressure (dP/dt).[17]

The purpose of this study was to determine the dynamics of morpho-functional and myocardial deformation characteristics of the left ventricle after revascularization in patients with CCS.

Materials and Methods

The study included 136 CCS patients of both sexes with stable anginal symptoms [(i) clinical scenario] and asymptomatic CAD at screening [(vi) clinical scenario].

The study protocol was reviewed and approved by the Ethics Committee of the Republican Specialized Centre of Cardiology. All participants provided the written informed consent.

Diagnosis of CCS was performed according to the 2019 ESC Guidelines for the diagnosis and management of chronic coronary syndromes.[1] Exclusion criteria were ACS, history of myocardial infarction within previous 3 months, valvular heart disease, heart failure with reduced ejection fraction, cardiac arrhythmia, renal impairment, severe co-morbidities.

The research protocol consisted of 3 stages. In the first stage (outpatient setting), all patients were subjected to general clinical examination and complex instrumental diagnostics, including 12-lead ECG, echocardiography, 2D-STE, stress echocardiography, and laboratory tests. In the second stage (hospital setting), coronary angiography was performed, and patients were divided into 3 groups based on the extent of coronary artery damage. The third stage (30-day follow-up examinations) included standard echocardiography and 2D-STE.

Transthoracic echocardiography (TTE)

Conventional 2D echocardiography was carried out according to the recommendations of the American Society of Echocardiography and the European Association of Cardiovascular Imaging[20] in M- and B-modes using the Siemens Acuson x700 (Korea, 2016) ultrasound machine. The LV volumes (LVEDV, LVESV) and LVEF were calculated using 1) the area–length method (using the apical 4-chamber and apical 2-chamber views) and 2) the modified Simpson's method (biplane method of disks) by tracing the endocardial border of LV cavity in both the apical four-chamber and two-chamber views in end-systole and end-diastole.

LV diastolic function was analyzed by measuring peak early diastolic filling (E) and late diastolic filling (A) velocities, E/A ratio. All parameters were obtained as the average of 5 consecutive cardiac cycles.

The values of LVEF (modified Simpson's method) were as follows:

•Normal range: 52% to 72% for men; 54% to 74% for women
•Mildly abnormal range: 41% to 51 for men; 41 to 53% for women
•Moderately abnormal range: 30% to 40% for men and women
•Severely abnormal range: <30% for men and women

Two-dimensional speckle tracking echocardiography (STE)

A common standard for STE is provided by a consensus document of the EACVI/ASE/Industry Task Force.[21] Two-dimensional images of 4-chamber, 3-chamber and 2-chamber apical views, as well as an LV parasternal short-axis view (at the root of papillary muscle), were recorded with the same ultrasound machine. Three consecutive cardiac cycle loops were recorded at end expiration. To ensure acceptable image quality, the frame rate was between 50 and 80 frames per second. A well-defined cardiac cycle was acquired for each view and stored digitally for offline analysis. All images were stored digitally and analyzed with offline software (Syngo Dynamics 9.0 software, Siemens Medical Solutions). Speckle tracking for myocardial strain was performed using Velocity Vector Imaging (VVI) software (TomTec-Arena TTA2, Germany). GLS and SR were automatically calculated as the average of 6 myocardial segments from 3 echocardiographic views.

Coronary angiography

CAG was performed via the Judkins technique through the femoral or radial artery access using Phillip Allura CV20 (Phillips Medical Systems, The Netherlands). The angiographical severity of coronary stenosis was assessed in the position with the most luminal narrowing, and the percentage of luminal narrowing was recorded according to the American Heart Association reporting system.[22] The SYNTAX score, an anatomical scoring system to grade the complexity of CAD, was calculated for each patient accordingly. All coronary lesions resulting in luminal narrowing of ≥50% in diameter for vessels ≥1.5 mm in diameter were considered significant stenosis. The SYNTAX score was calculated retrospectively according to the SYNTAX score algorithm.[23]

Statistical analysis was performed using the statistical software STATISTICA (v.10.0, StatSoft, USA). Baseline characteristics were summarized as frequencies and percentages for categorical variables and as mean ± standard deviation (SD) for continuous variables. Multiple comparisons were performed with one-way ANOVA with Tukey's pairwise comparisons. Student's paired t-test was used to compare the differences between the paired samples. Group comparisons with respect to categorical variables were performed using chi-square tests. A probability value of $P<0.05$ was considered statistically significant.

Results

A total of 100 patients with CCS were enrolled in the main group (MG) and underwent revascularization by PCI with intracoronary stenting using drug-eluting stents. Among the main-group patients, one-vessel, two-vessel, and three-vessel CAD were detected in 36(26.5%), 34(25%), and 30(22.0%) cases, respectively. The comparison group (CG) included 36 CCS patients with hemodynamically non-significant coronary lesions (<50% stenosis).

Comparative analysis of standard echocardiographic parameters revealed significant differences between MG and CG. LVEF values were within the normal range in all groups, with the highest value in the CG, followed by the one-, two- and three-vessel lesion groups. LVEF obtained by the area-length method and modified biplane Simpson's method did not differ (Table 1).

The assessment of the contractile function of the LV myocardium was also obtained by assessing the GLS and GLSR (Table 2). The comparative analysis of the LV myocardial deformation properties in the studied groups showed that less negative GLS and GLSR were found in the three-vessel CAD, followed by the two-vessel and one-vessel CAD groups, and CG. CG demonstrated more negative GLS and GLSR than all MG subgroups.

We analyzed the effect of revascularization on the GLS and found no statistically significant differences before and 48 hours after revascularization in all studied MG subgroups and CG. Thirty days after revascularization, GLS significantly showed more negative values in all MG subgroups: -18.12±0.63 versus -17.9±0.4 in one-vessel CAD, -16.13±0.71 versus -15.9±0.4 in two-vessel CAD and -13.91±1.25 versus -13.1±1.1 in three-vessel CAD. In CG with medical treatment only, GLS did not change statistically significantly but had more negative values than in the studied MG subgroups (Table 3).

Analysis of changes in LVEF after revascularization in the MG of patients with one-, two- and three-vessel CAD and in the CG after medical treatment did not reveal statistically significant dynamics (Figure 1).

Discussion

Currently, several studies have shown the advantages of GLS compared to LVEF in evaluating LV function, especially for mild systolic dysfunction.[24,25] Some studies have indicated that the use of 2D-STE aids in predicting recovery of myocardial contractile function after revascularization in CAD patients. A meta-analysis performed by Ballo et al.[26] evaluated the performance of 2D-STE for predicting the improvement of segmental LV contractile function after revascularization. The authors found that longitudinal strain (LS) and circumferential strain (CS) during low-dose dobutamine (LDD) stress provided equally high sensitivity and specificity for identifying reversible myocardial dysfunction, whereas LS and CS at rest showed lower accuracy. Wang et al.[27] evaluated LV function by 2D-STE and conventional echocardiography in 43 patients with coronary chronic total occlusion (CTO) who underwent PCI. The authors found that the GLS assessed with 2D-STE was improved as early as 1 day after CTO-PCI, whereas LVEF tended to improve up to 3 and 6 months after CTO-PCI.

In conclusion, the results indicate the absence of statistically significant changes in myocardial deformation indicators and morpho-functional parameters of the left ventricle in CCS patients 48 hours after revascularization. Thirty days after revascularization, GLS significantly improves, while LVEF remains unchanged. GLS is superior to LVEF in visualizing improvement in LV function after revascularization in patients with CCS.

Table 1.

Left Ventricular Systolic Function in CCS Patients.

Indicator	CG (n=36) [1]	One-vessel CAD (n=36) [2]	Two-vessel CAD (n=34) [3]	Three-vessel CAD (n=30) [4]	Statistics*
EDV, ml	91.3±14.7	112.7±22.7	110.8±24.4	104.6±22.3	F=7.3985 P=0.0001 P_{1-2}=0.0002 P_{1-3}=0.0011 P_{1-4}=0.0598 P_{2-3}=0.9821 P_{2-4}=0.4160 P_{3-4}=0.6504
ESV, ml	34.2±7.2	46.8±10.8	47.6±14.1	47.0±14.2	F=10.6695 P=0.0000 P_{1-2}=0.0001 P_{1-3}=0.0000 P_{1-4}=0.0001 P_{2-3}=0.9920 P_{2-4}=1.0000 P_{3-4}=0.9969
SV, ml	57.0±9.3	66.1±12.3	63.2±12.2	57.6±11.6	F=5.1933 P=0.0020 P_{1-2}=0.0051 P_{1-3}=0.1090 P_{1-4}=0.9965 P_{2-3}=0.7118 P_{2-4}=0.0160 P_{3-4}=0.2077
LVEF (area–ength), %	62.6±3.9	58.9±4.3	57.6±6.5	55.8±6.2	F=10.0022 P=0.0000 P_{1-2}=0.0184 P_{1-3}=0.0007 P_{1-4}=0.0000 P_{2-3}=0.7331 P_{2-4}=0.0875 P_{3-4}=0.5270
Biplane Simpson's LVEF (%)	63.9±3.7	57.8±5.6	57.3±8.1	55.0±8.7	F=10.9675 P=0.0000 P_{1-2}=0.0010 P_{1-3}=0.0004 P_{1-4}=0.0000 P_{2-3}=0.9895 P_{2-4}=0.3336 P_{3-4}=0.5209
Biplane Simpson's LVEDV, ml	98.6±17.3	115.3±30.4	111.8±31.7	101.8±27.3	F=2.9822 P=0.0337 P_{1-2}=0.0495 P_{1-3}=0.1823 P_{1-4}=0.9642 P_{2-3}=0.9495 P_{2-4}=0.1901 P_{3-4}=0.4596

**- one-way ANOVA with Tukey's pairwise comparisons.*

Table 2.

Assessment of LV myocardial function by 2D-STE in CCS patients.

Indicators	CG (n=36) [1]	One-vessel CAD (n=36) [2]	Two-vessel CAD (n=34) [3]	Three-vessel CAD (n=30) [4]	Statistics*
GLS, %	-21.0±0.7	-17.9±0.4	-15.9±0.4	-13.1±1.1	F=766.1250 P=0.0000 P_{1-2}=0.0000 P_{1-3}=0.0000 P_{1-4}=0.0000 P_{2-3}=0.0000 P_{2-4}=0.0000 P_{3-4}=0.0000
SR, c^{-1}	-2.1±0.1	-1.8±0.1	-1.5±0.2	-1.3±0.2	F=169.8468 P=0.0000 P_{1-2}=0.0000 P_{1-3}=0.0000 P_{1-4}=0.0000 P_{2-3}=0.0000 P_{2-4}=0.0000 P_{3-4}=0.0000

**- one-way ANOVA with Tukey's pairwise comparisons.*

Table 3.

The GLS changes in CCS patients after revascularization.

Group	Before PCI [1]	48 hours after PCI [2]	30 days after PCI [3]	*P*-value
CG	-21.0±0.70	-21.01±0.70	-21.03±0.71	NS
*One-vessel CAD	-17.9±0.4	-17.81±0.63	-18.12±0.63	0.015
*Two-vessel CAD	-15.9±0.4	-15.76±0.63	-16.13±0.71	0.024
*Three-vessel CAD	-13.1±1.1	-13.03±1.13	-13.91±1.25	0.014

**- statistically significant differences only between before and 30 days after revascularization.*

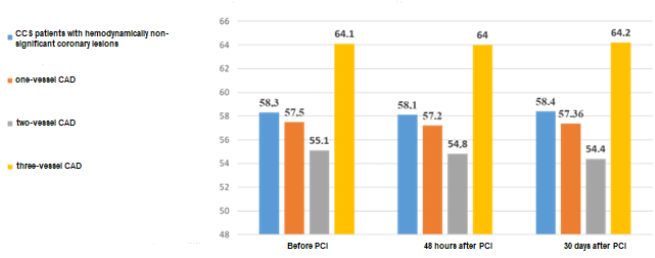

Fig. 2. *Changes in LVEF.*

Competing Interests

The authors declare that they have no competing interests.

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International Journal of Biomedicine 13(4) (2023) 246-249
http://dx.doi.org/10.21103/Article13(4)_OA2

ORIGINAL ARTICLE

Cardiology

INTERNATIONAL JOURNAL OF BIOMEDICINE

Earlobe Crease in Patients with Coronary Artery Disease at Hue Central Hospital

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Abstract

Background: The aim of this study was to evaluate the relationship between earlobe crease (ELC) and the severity of coronary artery disease (CAD).

Methods and Results: A cross-sectional descriptive study was conducted on 112 patients diagnosed with CAD who underwent coronary angiography at the Department of Emergency - Interventional Cardiology (Hue Central Hospital) from March 2023 to April 2023. All patients were examined clinically for bilateral ELC. Coronary artery injury was evaluated by using the Gensini score (GS). The results are presented as median (Me) and interquartile range (IQR [Q1-Q3]). A multiple logistic regression analysis was conducted to calculate the unadjusted and adjusted odds ratios (OR) with 95% CI.

Regarding ELC appearance, 84.8% of patients had bilateral ELC and 6.3% had unilateral ELC. As for length, 52.7% had complete length, while 38.4% had incomplete length. Regarding depth, the highest percentage of patients, 38.4%, had moderate depth, and the lowest percentage, 25%, had severe depth. Grade 2b had the highest frequency of 27.7%. GS of the bilateral group (28 [16–50]) was higher than that of the unilateral/no crease group (13 [7.5–21]), $P<0.05$. In terms of length, the GS of the complete group was significantly higher (32 [20–56]) than that of the incomplete group (16 [10–35]), $P<0.05$. Similarly, the GS of the group with severe depth was higher (33 [22.5–62]) than that of the mild/moderate depth group (21.5 [11.25–40]), $P<0.05$). In univariate analysis, bilateral crease, complete length, and severe depth were predictors of damage to ≥2 coronary arteries. Multivariate logistic regression analysis showed that all 3 factors above— bilateral appearance (OR=3.791, 95% CI: 1.306 to 11.009), complete length (OR=3.896; 95% CI: 3.896 to 9.103), and severe depth (OR=3.692; 95% CI: 1.173 to 11.620)—were independent prognostic factors for lesions of ≥2 coronary arteries.

Conclusion: ELC can be regarded as a clinical sign suggesting the patient should be considered for CAD screening and prognosis.**(International Journal of Biomedicine. 2023;13(4):246-249.)**

Keywords: earlobe crease • coronary artery disease • coronary angiography

For citation: Doan CT, Tran KN. Earlobe Crease in Patients with Coronary Artery Disease at Hue Central Hospital. International Journal of Biomedicine. 2023;13(4):246-249. doi:10.21103/Article13(4)_OA2

Abbreviations

CAD, coronary artery disease; **DELC**, diagonal ELC; **ELC**, earlobe crease; **GS,** Gensini score.

Introduction

Coronary artery disease (CAD) is the leading cause of disability and death worldwide. According to WHO 2019, ischemic heart disease was the leading cause of death, accounting for 16% of total deaths globally; deaths have increased from 2 million to 8.9 million from 2000 to 2019.[1] In Vietnam, myocardial infarction is one of the highest causes of death in the country.[2] The cost for each hospital admission of myocardial infarction in Vietnam is higher than the gross domestic product per capita.[3] In addition, in developing countries, cardiovascular disease tends to affect younger patients and thus negatively impacts society regarding resources and economic efficiency.

The most common cause of CAD is atherosclerosis, associated with cardiovascular risk factors such as smoking, obesity, sedentary lifestyle, hypertension, diabetes, and dyslipidemia. The diagnosis of CAD is based on clinical presentation and diagnostic test results. However, the choice of diagnostic tests depends crucially on the experience of

medical staff and facilities. Faced with a heavy burden of disease on society, high treatment costs for the family, and severe sequelae affecting the patient's quality of life, it is not only a matter of controlling risk factors well but also finding the right healthcare solutions. Clinical signs or symptoms suggestive of CAD also contribute a significant benefit.

The DELC is a 45-degree diagonal groove that runs from the ridge of the ear canal through the lobe and ends at the free edge of the earlobe. It was first described by American physician Frank in 1973 in the New England Journal of Medicine in a series of patients with CAD.[4] Since then, there have been many published reports on the association between earlobe imprinting and atherosclerosis, especially CAD. Several studies have reported earlobe crease (ELC) as an early warning sign of CAD, atherosclerosis,[5] peripheral vascular disease,[6] and cerebrovascular disease.[7,8] Realizing that this is a simple clinical sign, easy to detect and related to the prediction of CAD, helps to manage health more effectively, especially at health facilities with limited resources. We conducted the present study to evaluate the relationship between ELC and the severity of CAD.

Materials and Methods

A cross-sectional descriptive study was conducted on 112 patients diagnosed with CAD who underwent coronary angiography at the Department of Emergency - Interventional Cardiology (Hue Central Hospital) from March 2023 to April 2023.

Inclusion criteria: patients 18 years of age or older with a diagnosis of CAD with significant coronary stenosis (≥50%) of the lumen in at least one of the 3 main coronary vessels (left anterior descending artery [LAD], left circumflex artery [LCX], right coronary artery [RCA])[9] or ≥50% stenosis of the left main coronary artery.

Exclusion criteria: patients who did not consent to participate in the study, patients with severe trauma, patients with previous percutaneous coronary intervention or coronary artery bypass graft surgery, patients with myocardial bridge or cardiomyopathy, and patients with damage to the earlobe.

All patients were examined clinically for bilateral ELC. The following features on both the right and left earlobes were noted:[10]

Length: complete (the mark runs through the entire earlobe), incomplete (the mark runs through the earlobe but does not reach the outer margin).

Depth: mild (vaguely insinuated), moderate (a fold where the base of the sulcus can be observed), severe (the base of the sulcus cannot be seen).

Classification of ELC:

Grade 1: some wrinkles on the earlobe

Grade 2a: creases running over more than half of the earlobe

Grade 2b: shallow crease running through the entire earlobe

Grade 3: deep folds running through the entire earlobe

Then, we noted the common characteristics of ELC for both ears based on the ear with more severe and obvious damage. We evaluated coronary artery injury by using the Gensini score (GS).[11]

Statistical analysis was performed using the statistical software package SPSS version 21.0 (SPSS Inc, Armonk, NY: IBM Corp). The results are presented as median (Me) and interquartile range (IQR [Q1-Q3]). The Kruskal-Wallis test was used to compare the medians of the two groups. A multiple logistic regression analysis was conducted to calculate the unadjusted and adjusted odds ratios (OR) with 95% confidence intervals (95% CI). The frequencies of categorical variables were compared using a chi-square test. A probability value of $P<0.05$ was considered statistically significant.

Results

The study population comprised 112 patients diagnosed with CAD who underwent coronary angiography. Regarding appearance, 84.8% of patients had bilateral ELC and 6.3% had unilateral ELC. As for length, 52.7% had complete length, while 38.4% had incomplete length. Regarding depth, the highest percentage of patients, 38.4%, had moderate depth, and the lowest percentage, 25%, had severe depth. Grade 2b had the highest frequency of 27.7% (Table 1). GS of the bilateral group was higher [28 (16–50)] than that of the unilateral/no crease group (13 [7.5–21]), $P<0.05$ (Table 2). In terms of length, the GS of the complete group was significantly higher (32 [20–56]) than that of the incomplete group (16 [10–35]), $P<0.05$ (Table 3). Similarly, the GS of the group with severe depth was higher (33 [22.5–62]) than that of the mild/moderate depth group (21.5 [11.25–40]), $P<0.05$ (Table 4). In univariate analysis, bilateral crease, complete length, and severe depth were predictors of damage to ≥2 coronary arteries (Table 5). Multivariate logistic regression analysis showed that all 3 factors above— bilateral appearance (OR=3.791; 95% CI: 1.306 to 11.009), complete length (OR=3.896; 95% CI: 3.896 to 9.103), and severe depth (OR=3.692; 95% CI: 1.173 to 11.620)—were independent prognostic factors for lesions of ≥2 coronary arteries (Table 6).

Table 1.

General characteristics of both ears in the study subjects.

ECL		n	%
Appearance	Bilateral	95	84.8
	Unilateral	7	6.3
	No crease	10	8.9
Length	Complete	59	52.7
	Incomplete	43	38.4
	No crease	10	8.9
Depth	Mild	31	27.7
	Moderate	43	38.4
	Severe	28	25
	No crease	10	8.9
Grade	1	17	15.2
	2a	26	23.2
	2b	31	27.7
	3	28	25
	No crease	10	8.9

Table 2.

Distribution of the number of damaged coronary artery branches and GS with the presence of ELC.

Lesion branches	ELC				*P*-value
	Bilateral		Unilateral / No crease		
	n	%	n	%	
1 branch	26	27.4	10	58.8	0.01
≥ 2 branches	69	72.6	7	41.2	
GS, Me (Q1;Q3)	28 (16–50)		13 (7.5–21)		<0.05

Table 3.

Distribution of the number of damaged coronary artery branches and GS with length of ELC.

Lesion branches	ELC				*P*-value
	Complete		Incomplete / No crease		
	n	%	n	%	
1 branch	11	18.6	25	47.2	<0.01
≥ 2 branches	48	81.4	28	52.8	
GS, Me (Q1;Q3)	32 (20–56)		16 (10–35)		<0.05

Table 4.

Distribution of the number of damaged coronary artery branches and GS with depth of ELC.

Lesion branches	ELC				*P*-value
	Severe		Mild/ Moderate/ No crease		
	n	%	n	%	
1 branch	4	14.3	32	38.1	<0.02
≥ 2 branches	24	85.7	52	61.9	
GS, Me (Q1;Q3)	33 (22.5–62)		21.5 (11.25– 40)		<0.05

Table 5.

Univariate logistic regression: associations between risk factors and ELC classification with lesions ≥ 2 coronary arteries.

Variable	OR	95% CI	*P*-value
Sex (male)	2.566	0.692 to 9.513	>0.05
Age (≥ 70)	0.633	0.233 to 1.716	>0.05
Obesity	1.376	0.512 to 3.679	>0.05
Hypertension	3.114	0.922 to 10.513	>0.05
Diabetes	1.211	0.446 to 3.291	>0.05
Dyslipidemia	1.821	0.678 to 4.886	>0.05
Smoking	0.369	0.113 to 1.382	>0,05
Bilateral ELC	4.862	1.474 to 16.036	<0.05
Complete length	4.727	1.781 to 12.548	<0.05
Severe depth	5.176	1.467 to18.266	<0.05

Table 6.

Multivariate logistic regression: associations between ELC classification and lesions ≥ 2 coronary arteries

Characteristics	OR	95% CI	*P*-value
Bilateral ELC	3.791	1.306 to 11.009	<0.05
Complete length	3.896	3.896 to 9.103	<0.05
Severe depth	3.692	1.173 to 11.620	<0.05

Discussion

Regarding the number of lesion branches, the lesion of 2 branches and or more in each feature of the ELC can be seen in the group with a bilateral crease at 72.6%, in the group with complete length at 81.4%, and the group with a severe depth at 85.7%.

Comparison between each subgroup of characteristics of the crease with the number of damaged coronary artery branches showed that in lesions of 2 branches and more, the group with bilateral ELC was higher than the unilateral/no crease group, 72.6% and 41.2%, respectively; the difference is statistically significant (P<0.05). The median GS of the bilateral group was significantly higher at 28 points than that of the unilateral/no crease group at 13 points (P<0.05). Similarly, the percentage of the complete length group with lesions of 2 branches and more, 81.4%, was higher than that of the incomplete/no crease group, 52.8%, and the median GS of the 2 groups was 32 and 16 points, respectively; this difference is statistically significant (P<0.05). And finally, about crease depth, patients with severe depth had a significantly higher percentage of lesion in 2 branches, 85.7%, than those with moderate/mild/no crease, 61.9% (P<0.05), and the median GS was 33 versus 21.5 points, respectively.

Compared with a study by Wu et al.,[12] the rate of CAD and the rate of damaged 2 vessels in the group with a crease was significantly higher than in the group without a crease. A study performed by Arefi et al.[13] also showed similar results with the lesions of 2 branches and 3 branches of the coronary artery in the group with a crease significantly higher than the group without a crease (P<0.05). Thilo et al.[14] showed that the rate of 1- and 2-vessel lesions in the crease group with grade 2/3 was higher than in the group with grade 0/1, but the rate of 3-vessel lesions between 2 grades differed. Kahyaoglu et al.[15]showed that the median value of GS in the group with a crease was higher than the group without a crease, and the difference was statistically significant (P<0.05).

In general, the results of our study are quite similar to the above studies, and show that subjects with multivessel CAD have a higher rate of CAD related to ELC. When we tested the predictability of multivessel CAD of the ELC patients by logistic regression, it was found that the bilateral crease, the complete length, and the severe depth could predict damage of multivessel CAD, independent of other risk factors, with ORs of 4.862 (95% CI: 1.474 to 16.036), 4.727 (95% CI: 1.781 to 12.548) and 5.176 (95% CI: 1.467 to 18.266), respectively, when performed with the model including male, age ≥70, obesity, hypertension, diabetes, and smoking (P<0.05).

Compared with the study of Shmilovich et al. on 430 subjects with coronary angiography, the results of logistic regression are that the ELC can predict damage to 2 vessels and more with OR of 1.9 (95% CI: 1.2 to 3.1) when adjusted for age >70 years, male sex, smoking, hypertension, diabetes mellitus, dyslipidemia, and any chest pain symptoms (P<0.05).[16]

In conclusion, ELC can be regarded as a clinical sign suggesting the patient should be considered for CAD screening and prognosis.

Competing Interests

The authors declare that they have no competing interests.

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International Journal of Biomedicine 13(4) (2023) 250-254
http://dx.doi.org/10.21103/Article13(4)_OA3

ORIGINAL ARTICLE

Obstetrics and Gynecology

INTERNATIONAL JOURNAL OF BIOMEDICINE

Outcomes of Frozen-Thawed Embryo Transfer in Overweight and Obese Infertile Women

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Abstract

***Background*:** This study aimed to retrospectively analyze the effects of different endometrial preparation protocols on live birth rate (LBR) of overweight/obese patients in frozen-thawed embryo transfer (FET) cycles.

***Methods and Results*:** The study population comprised women who received IVF treatment between June 2013 and December 2020 in the Reproductive Medical Centre. We collected 529 patients with BMI ≥24 kg/m^2 according to the inclusion criteria. The patients were divided into four groups according to the different endometrial preparation protocols in the freeze-thaw cycle: modified natural cycle group (135 cases), stimulated cycle group (124 cases), artificial cycle group (85 cases), and artificial cycle with down-regulation group (185 cases). There was no statistical difference in LBR, clinical pregnancy rate, miscarriage rate, or ectopic pregnancy rate among different endometrial preparation protocols. Multivariate logistic regression analysis showed that only the duration of infertility was related to the LBR (P=0.048).

Conclusion: For overweight and obese people who want to receive FET, different endometrial preparation protocols may not affect the LBR.**(International Journal of Biomedicine. 2023;13(4):250-254.)**

Keywords: frozen-thawed embryo transfer • live birth rate • overweight • obesity

For citation: Zhang Y. Outcomes of Frozen-Thawed Embryo Transfer in Overweight and Obese Infertile Women. International Journal of Biomedicine. 2023;13(4):250-254. doi:10.21103/Article13(4)_OA3

Abbreviations

AC, artificial cycle; **AFC**, antral follicular count; **CET**, cryopreserved embryo transfer; **FET**, frozen-thawed embryo transfer; **GnRH**, gonadotrophin-releasing hormone; **hCG**, human chorionic gonadotropin; **hMG**, human menopausal gonadotropin; **IVF**, in vitro fertilization; **ICSI**, intracytoplasmic sperm injection; **LH**, luteinizing hormone; **LBR**, live birth rate; **MC**, menstrual cycle; **OPU**, ovum pick up.

Introduction

With the development of the economy and the improvement of living standards, obesity has become a world health problem and is a major public health issue in China. Overweight and obesity have increased rapidly in the past four decades.[1,2] The overweight rate and obesity rate of women of childbearing age in China have reached 25.4% and 9.2%, respectively.[3] Obesity affects female reproduction in that obese women show lower levels of gonadotropin hormones, reduced fecundity, higher miscarriage rates, and poorer outcomes of in vitro fertilization.[4] There is also an association between maternal overweight/obesity and gestational diabetes, hypertensive disorders, intrauterine fetal death, stillbirth, and neonatal mortality.[5] Overweight/obese patients have difficulty in losing weight.

Endometrial preparation protocols in the frozen-thawed embryo transfer (FET) cycle can be divided into natural cycle, modified natural cycle, ovulation stimulation cycle, and artificial protocol with or without pretreatment of GnRH-agonist. There is no conclusion on which is better for endometrial cycle preparation.[6] A study by Gu et al.[7] shows that compared with hormone replacement therapy, the natural

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cycle FET of young women with normal menstrual cycle (MC) has improved the live birth rate (LBR).

Our study posed the question, which endometrial preparation protocol is more suitable for obese patients? For this article, we retrospectively analyzed the LBR of different FET protocols for overweight/obese patients.

Methods

Study Design and Population

In our retrospective study, the study population comprised women who received IVF treatment between January 2013 and December 2020 in the Reproductive Medical Centre, Beijing CHAO-YANG Hospital, Capital Medical University, Beijing. All the patients received follow-up observation for at least one year, and study data were extracted from the medical record. The study was approved by the ethics committee of the hospital (LGH-2022-No-4). Because of the retrospective character of the study, informed consent was waived.

Patients were considered eligible if they met the following criteria: women who had previous IVF/ICSI cycles with embryo cryopreservation, BMI ≥24 kg/m^2, age of 20–42, regular MCs (25–35 days), first cycle of FET between June 2013 and December 2020, and at least one high-quality embryo was transferred. The exclusion criteria were endometriosis, history of recurrent pregnancy loss, uterine pathology, problems of the immune system and abnormal coagulation mechanism, rescued ICSI for routine IVF fertilization failure, TESA-ICSI due to male factor, or cycles that were canceled due to failure of embryo thaw survival.

Endometrial Preparation Protocols

Modified natural cycle protocol: The patients underwent transvaginal ultrasound on Days 8 to 10 of the MC. Follicular growth was monitored through transvaginal ultrasound, and luteinizing hormone (LH) was measured in urine or serum. When the leading follicle had reached a mean diameter of >17mm, urine test paper was positive, or serum LH level was 20 IU/L, 250 ug recombinant hCG was administered to trigger ovulation. The day of ovulation was confirmed by transvaginal ultrasound. From the day of ovulation, dydrogesterone 20 mg daily was initiated. The FET was scheduled on Day 3 (cleavage stage) or Day 5 (blastocyst stage).

Stimulated cycle: Patients were administered letrozole (Jiangsu Hengrui) 2.5 mg/d on Days 3 to 5 of the MC for five consecutive days or hMG 37.5 IU to 7 5IU on Days 3 to 5 of the MC for five consecutive days. Follicular development was monitored using ultrasonography. When the diameter of dominant follicles was ≥18 mm, and endometrial thickness ≥7 mm, 250 ug recombinant hCG was administered to trigger ovulation, and dydrogesterone 20 mg daily was initiated. Embryos were transferred 3 to 5 days later.

Artificial cycle (AC) and down-regulated AC: Endometrial preparation was initiated with oral estradiol valerate (Progynova; Bayer Schering Pharma AG) at a daily dose of 6-8 mg from Day 2 to 3 of the MC with or without down-regulation of GnRH agonist. The serum estradiol, LH, and progesterone levels were measured, and a transvaginal ultrasound was performed 10 to 12 days later. Provided the endometrial thickness reached 7mm or more, and the serum progesterone level was <1.5 ng/ml, 60 mg of intramuscular progesterone was initiated. Embryos were transferred 4 or 6 days later.

Luteal phase support and pregnancy confirmation: From the day of embryo transfer, luteal phase support was continued. The pregnancy testing was performed by measuring serum β-hCG 14 days after embryo transfer. If the β-hCG level was >15 IU/L, biochemical pregnancy was diagnosed, and luteal support was continued. All the patients were followed until one year after embryo transfer.

Observation Indicators

Main outcome measure: LBR. Secondary outcome measures: clinical pregnancy rate, miscarriage rate, ectopic pregnancy. Clinical pregnancy was defined as a pregnancy diagnosed by ultrasonographic visualization of the gestational sac, fetal bud, and fetal heart at four weeks after transfer. Ectopic pregnancy was defined as a gestational sac observed by ultrasound outside the uterine cavity. Miscarriage was defined as a spontaneous loss of a clinical pregnancy before 28 completed weeks of gestational age. Live birth was defined as the delivery of at least one live-born baby beyond 28 weeks of gestation. The birth rate was defined as live deliveries (at least one live birth) per woman after embryo transfer.

Statistical analysis was performed using the statistical software package SPSS version 21.0 (SPSS Inc, Armonk, NY: IBM Corp). The normality of distribution of continuous variables was tested by the Kolmogorov-Smirnov test with the Lilliefors correction and Shapiro-Wilk test. For the descriptive analysis, results are presented as mean (M) and standard deviation (SD). For data with normal distribution, inter-group comparisons were performed using Student's t-test. Multiple comparisons were performed with one-way ANOVA or a non-parametric Kruskal-Wallis test. Group comparisons with respect to categorical variables were performed using the chi-square test. Binary logistic regression analysis was used to assess the association between the type of endometrial preparation and LBR. A multiple logistic regression analysis was conducted to calculate the unadjusted and adjusted odds ratios (OR) with 95% confidence intervals (CI). A probability value of P<0.05 was considered statistically significant.

Results

We collected 529 patients with BMI ≥24 kg/m^2 according to the inclusion criteria from 1/1/2013 to 12/31/2020. The patients were divided into four groups according to the different endometrial preparation protocols in the freeze-thaw cycle: modified natural cycle group (135 cases), stimulated cycle group (124 cases), artificial cycle group (85 cases), and artificial cycle with down-regulation group (185 cases). There were statistical differences among the four groups in the AFC and endometrial pattern (Tables 1 and 2). There was no statistical difference in LBR, clinical pregnancy rate, miscarriage rate, or ectopic pregnancy rate among different endometrial preparation protocols.

The endometrial preparation plan is divided into two groups according to whether there is corpus luteum. The

modified natural cycle group and stimulated cycle group are groups with luteal, and the artificial cycle group with or without down-regulation are groups without luteal. There was no statistical difference in clinical pregnancy rate, LBR, miscarriage rate, or ectopic pregnancy rate between the groups with or without corpus luteum.

Table 1.

Characteristics of the patients at baseline.

Group	MNC	SC	AC	AC with GnRHa	*P*
Number	135	124	82	185	
Age at OPU	32.39(6)	33.11(6)	32.36(5)	32.48(5)	0.74
Age at embryo transfer	32.65(7)	33.53(6)	32.89(5)	33.13(5)	0.84
BMI, kg/m^2	25.53(2.88)	26.2(2.91)	25.71(3.08)	26.35(3.42)	0.14
Primary infertility, %	66(48.9)	60(48.4)	46(56.1)	111(60.0)	0.12
Duration of infertility (years)	3.00(3)	3.36(2)	2.00(3)	3.00(3)	0.51
Infertility cause					0.71
Tubal factors	62(45.9)	60(48.4)	42(51.2)	100(54.1)	
Male factor	23(17.0)	46(15.3)	4(4.9)	21 (11.4)	
Other factors	32(23.7)	25(20.2)	29(35.4)	44 (23.8)	
Mixed factors	18(13.3)	20(16.1)	7(18.5)	20 (10.8)	
bFSH	6.49(2)	6.12(2)	5.78(2)	5.96 (2)	0.18
AFC	13(7)[a]	15(8)	17(11)[a]	14 (11)[a]	0.024
Protocol of COH (n, %)					0.21
Antagonist	81(75.7)	66(53.2)	54(65.9)	94 (50.8)	
Agonist	38(28.1)	45(36.3)	20(24.4)	71 (38.4)	
Others	16(11.9)	13(10.5)	9(9.8)	20 (10.8)	
Fertilization type (n, %)					0.11
IVF	96(71.1)	88(71.0)	63(76.8)	134 (72.4)	
ICSI	22(16.3)	9(7.3)	10(12.2)	26 (14.1)	
IVF/ICSI	17(12.6)	27(21.8)	9(11.0)	25 (13.5)	

MNC - modified natural cycle, SC - stimulated cycle, AC -artificial cycle; [a] The difference between groups is statistically significant.

Univariate logistic regression analysis showed that age at OPU, age at CET, and duration of infertility were correlated with LBR (P<0.05) (Table 3). There was no correlation between the LBR and the endometrial preparation protocol, BMI, ovarian hyperstimulation protocol, fertilization method, endometrial thickness and morphology, the number of transferred embryos, or high-quality embryos in the freeze-thaw cycle. Multivariate logistic regression analysis showed that only the duration of infertility was related to the LBR (P=0.048), correcting age at OPU, age at CET, AFC, endometrial pattern, and endometrial preparation plan. According to the stratified analysis of BMI, there was no correlation between the LBR and the endometrial preparation protocol.

Table 2.

Outcomes of different endometrial preparation protocol.

Group	MNC	SC	AC	AC with GnRHa	P
Number	135	124	82	185	
Endometrial thickness	10(2.5)	10(2.9)	9(2.5)	10(2.6)	0.12
Endometrial pattern					0.011
A	69(51.1)[b]	60(48.4)[b]	49(59.8)[b]	121(65.4)	
others	66(48.9)[b]	64(51.6)[b]	33(40.2)[b]	64(34.6)	
Type of embryo transferred					0.25
D3	123(91.1)	105(84.7)	64(78.0)	160(86.5)	
Blastosphere	10(7.4)	17(13.7)	16(19.5)	23(12.4)	
Both	2 (1.5)	2(1.6)	2(1.6)	2(1.1)	
No. of embryos transferred (n, %)					0.34
1	10(7.4)	13(10.5)	11(13.4)	14(7.6)	
2	125(92.6)	111(89.5)	71(86.6)	171(92.4)	
No. of good quality embryos transferred (n,%)					0.39
1	85(63)	80(64.5)	55(61.1)	106(57)	
2	50(37)	44 (35.5)	27(32.9)	79(42)	
Clinical pregnancy rate, %	53.3	62.1	56.1	58.4	0.54
Live birth rate, %	40.7	45.2	48.8	45.4	0.69
Miscarriage rate, %	11.9	14.5	6.1	11.9	0.32
Ectopic pregnancy rate, %	0	0.8	0	1.1	0.53

MNC - modified natural cycle, SC - stimulated cycle, AC -artificial cycle; [b] There was no statistical difference between groups b1, 2, and 3, but there was a statistical difference compared to the four groups.

Table 3.

Univariate analysis for LBR.

Covariate	OR	(95% CI)	*P*
Age at OPU	0.948	0.908-0.989	0.015
Age at embryo transfer	0.950	0.910-0.992	0.019
BMI (kg/m^2)	0.960	0.897-1.027	0.233
Infertility type			
Primary infertility	Reference		
Secondary infertility	0.656	0.463-0.928	0.017
Duration of infertility (years)	0.918	0.856-0.985	0.017
Infertility cause			
Tubal factors	Reference		
Male factor	1.070	0.991-2.917	0.054
Other factors	1.043	0.682-1.595	0.846
Mixed factors	1.257	0.729-2.167	0.411
bFSH	0.972	0.897-1.054	0.499
AFC	1.032	1.000-1.047	0.054

Table 3 (Continued).

Univariate analysis for LBR.

Covariate	OR	(95% CI)	*P*
Protocol of COH (n, %)			
Antagonist	Reference		
Agonist	1.221	0.837-1.78	0.301
Others	0.788	0.426-1.46	0.449
Fertilization type (n, %)			
IVF	Reference		
ICSI	0.82	0.484-1.391	0.462
IVF/ICSI	1.042	0.639-1.698	0.870
Endometrial thickness	1.056	0.971-1.149	0.200
Endometrial pattern			
A	Reference		
others	0.957	0.676-1.354	0.802
Type of embryo transferred			
D3	Reference		
Blastosphere	1.375	0.820-2.307	0.227
Both	1.294	0.32-5.24	0.718
No. of embryos transferred (n, %)			
1	Reference		
2	1.529	0.824-2.837	0.178
No. of good quality embryos transferred (n, %)			
1	Reference		
2	1.055	0.741-1.503	0.766

Discussion

This paper reviewed and analyzed the effect of different freeze-thaw cycles on the LBR in overweight and obese women. The results showed no statistical difference in the LBR between different endometrial preparation protocols. The factors affecting the LBR were age at OPU, age at CET, and duration of infertility. These results will have some clinical implications for the transfer of frozen embryos to overweight and obese individuals.

The pre-pregnancy overweight/obese population is associated with adverse perinatal pregnancy outcomes, such as pregnancy diabetes, pre-eclampsia, induced labor, cesarean section, preterm birth, and macrosomia. Pre-pregnancy overweight and obesity are associated with obesity in children and adolescents. A meta-analysis showed that even 5% to 7% weight loss may improve metabolic health and pregnancy outcomes.[8] Obese patients have difficulty in losing weight and easily rebound. And even if weight loss is successful, obesity still impacts physical health. There is no report in the literature on what the best endometrial preparation protocol for overweight and obese patients is. Our results provide a clinical basis for guiding the embryo development of such populations.

In the past few years, the use of FET has increased exponentially. It is very important to optimize the endometrial preparation protocol before FET to improve the pregnancy outcome. There are five kinds of freeze-thaw cycle endometrial preparation protocols. Natural cycle protocol is the closest to the physiological situation. Estrogen produced by follicular development promotes the growth of endometrium. After waiting for the follicle to mature naturally and ovulation, the embryo is transferred according to the embryo development date. The modified natural cycle is to give hCG triggers to promote ovulation when the follicles mature. The stimulation cycle or ovulation induction cycle is the use of letrozole, or combined with gonadotropin, to induce the development of single or multiple follicles, and hCG triggers ovulation when the follicles mature. In the natural cycle, modified natural cycle, and stimulation cycle, the luteal support of the embryo depends on the luteal function formed in the cycle, and progesterone supplemented externally is small. These protocols use fewer drugs, and the time should be determined according to the follicular growth. Patients have more visits, and the cycle cancelation rate is high.

In the AC, the growth of the endometrium entirely depends on exogenous estrogen. After the endometrium reaches a certain thickness, exogenous progesterone is added to transform the endometrium, and the corpus luteum support is continued until the placenta is formed after embryo transfer. The AC with down-regulation is pretreated with GnRH-agonist. Whether the AC is pretreated with GnRH-agonist or not, there is no luteal formation. The artificial protocol is relatively reliable and convenient for work arrangements.

Many scholars have studied and compared the advantages and disadvantages of different endometrial preparation protocols. Some patients with ovulation disorders are not suitable for natural cycles or modified natural cycle protocols. If it is difficult to induce ovulation, only AC schemes can be used. AC may increase the risk of eclampsia during pregnancy.[7] According to a recent meta-analysis, GnRH-agonist pretreatment in FET can improve implantation, clinical pregnancy, and LBR, especially in patients with repeated implantation failure. GnRH-agonist pretreatment seems to improve the results of FET, although its premature delivery rate is higher.[9] The latest large European register study evaluated early pregnancy complications and LBR per pregnancy after FET between three different cycle regimens. The miscarriage rate was highest in the AC, compared with the stimulation or natural cycles.[10] The latest review showed no strong evidence to support the use of one preparation method over the other regarding pregnancy outcomes.[6]

The results of the analysis suggest that the factors related to the LBR are age and duration of infertility; that is, young patients and shorter duration of infertility are more likely to obtain live birth. Many studies have shown that maternal age was associated with poor pregnancy outcomes, such as miscarriage, preterm birth, stillbirth, low birth weight, neonatal death, and perinatal neonatal death.[11] As age increases, the number and quality of eggs decrease. Age also has an impact on the endometrium[12] and a crucial impact on assisted reproductive survival.[12-14] The longer the duration of

infertility, the more severe it is and the more difficult it is to achieve a live birth. For overweight and obese patients, the FET protocol does not affect the biochemical pregnancy rate, clinical pregnancy rate, or LBR.[15]

Limitations of the Study

The number of cases was limited. The analysis results may be more reliable if combined with data from multiple centers. This article was a retrospective study. The research results need to be further confirmed by designing randomized controlled studies. The outcome data of clinical complications in our research data are incomplete. Unfortunately, we could not analyze whether different endometrial preparation protocols increase pregnancy complications.

Conclusion

For overweight and obese people who want to receive FET, different endometrial preparation protocols may not affect the LBR. Age and duration of infertility were related to LBR. Weight loss is recommended before accepting FET. Further research needs to be confirmed by a prospective randomized controlled study.

Competing Interests

The author declared that there are no competing interests.

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International Journal of Biomedicine 13(4) (2023) 255-260
http://dx.doi.org/10.21103/Article13(4)_OA4

ORIGINAL ARTICLE

Obstetrics and Gynecology

INTERNATIONAL JOURNAL OF BIOMEDICINE

The Immune Profile of the Endometrium in the "Uterine Factor" of Infertility

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Abstract

Background: This study aimed to investigate the endometrial characteristics (pathomorphological and immunological) of women with infertility.

Methods and Results: Data from an immunohistochemical study of endometrial biopsies (TNF-α, IL-10, GM-CSF, CXCL16, BCA1, TGF-β1) collected during the "implantation window" and microbiota studied by real-time polymerase chain reaction in 171 patients (21 women with unexplained infertility, 36 - chronic endometritis, 74 - tubal-peritoneal infertility, 22 - external genital endometriosis, 8 - "thin" endometrium, and 10 healthy fertile women from the comparison group) were analyzed to identify molecular signatures. Chronic endometritis was verified morphologically and immunohistochemically.

Each group revealed different immune endometrial phenotypes. The basis of the "normal" phenotype was a controlled immune inflammation and a *Lactobacillus*-dominant microbiota (LDM) type. In contrast to the comparison group, in the group with the phenotype of chronic inflammation, an excessive immune response (overexpression of TNF-α, GM-CSF, CXCL16, BCA1, and a decrease in IL-10 and TGF-β1 in glandular epithelium and stroma) was determined on the background of non-*Lactobacillus*-dominated microbiota (NLDM) type (63.3%) (P<0.001). The peculiar feature of a dysplastic phenotype was a "poor" immune response, with maximal TGF-β1 overexpression (P<0.001) and a NLDM type (47.1%). We determined an excessive immune response in the proliferative endometrial phenotype (GM-CSF overexpression by 1.2 times in the glandular epithelium and stroma [P<0.001 in both cases] and a decrease in IL-10 by 1.6 times in the glandular epithelium and 1.2 times in the stroma [P<0.001 in both cases]). Uterine microbiome disorders were detected less frequently than in patients with the inflammation phenotype (31.6%) (P=0.01). In the phenotype with impaired immune status, there was a decrease in GM-CSF, BCA1, CXCL16, TNF-α, and IL-10 markers in both endometrial compartments (P<0.001) with a LDM type (81.2%).

Conclusion. The molecular signatures of the endometrium are due to the heterogeneity of immune factors and microbiota. Aberrant expression of immune factors may contribute to the formation of a microenvironment unfavorable for blastocyst implantation.**(International Journal of Biomedicine. 2023;13(4):255-260.)**

Keywords: infertility • implantation window • molecular phenotype • cytokines • endometrium

For citation: Polina ML, Radzinsky VE, Mikhaleva LM, Vityazeva II, Khamoshina MB, Mikhalev SA, Douglas NI. The Immune Profile of the Endometrium in the "Uterine Factor" of Infertility. International Journal of Biomedicine. 2023;13(4):255-260. doi:10.21103/Article13(4)_OA4

Abbreviations

CE, chronic endometritis; **EP**, endometrial polyp; **EGE**, external genital endometriosis; **H-score**, Histo-score; **LDM**, *Lactobacillus*-dominated microbiota; **NLDM**, non-*Lactobacillus*-dominated microbiota; **RT-PCR**, real-time polymerase chain reaction; **TPI**, tubal-peritoneal infertility; **UI**, unexplained infertility.

Introduction

Over the past three decades, progress has been made in understanding the mechanisms of interaction between the endometrium and a genetically and immunologically distinct embryo. The immunological dialogue during the "implantation window" occurs under conditions of decidual transformation of fibroblast-like cells of the endometrial stroma.[1] The number, type, and activity of immune cells in intercellular signaling during endometrial remodeling, decidualization and implantation of blastocysts is regulated by ovarian steroid hormones, 17ß-estradiol and progesterone.[2] The receptive endometrium during the "implantation window" is capable of expressing cytokines, chemokines, growth factors, and adhesion molecules that contribute to the creation of an inflammatory environment and trophoblast migration.[3] The heterogeneity of data on the features of the immune system regulation leading to infertility and implantation failures is associated with complex interactions of molecular mediators during the "implantation window."

Most studies are limited to stating a violation of the optimal proinflammatory immune environment for implantation; however, the role of the microbiota in the reactions of the interaction of the endometrium-local immunity system is poorly understood.(4) The development of gene sequencing technology made it possible to determine the microbial composition of the uterus, whose functional interactions with the endometrium are different in physiological status and diseases.[5] Most studies distinguish "Lactobacillus-dominated microbiota" (LDM) type (>90% of lactobacilli) and "non-*Lactobacillus*-dominated microbiota" (NLDM) (<90% of lactobacilli, >10% of other bacteria).[6] The molecular mechanisms of the influence of microbial diversity on the blastocyst implantation process remain unclear due to the paucity and inconsistency of data on the regulation of endometrial remodeling and receptivity.[7]

This study aimed to investigate the endometrial characteristics (pathomorphological and immunological) of women with infertility.

Material and Methods

We performed a prospective examination of 171 women of reproductive age with infertility, including after ineffective attempts of in vitro fertilization. The following groups were identified: 21 patients with unexplained infertility (UI) (Group UI), 36 patients with chronic endometritis (CE) (Group CE), 74 patients with tubal-peritoneal infertility (TPI) (Group TPI), 22 patients with external genital endometriosis (EGE) (Group EGE), and 8 patients with "thin" endometrium (TE) (Group TE). The comparison group consisted of 10 healthy fertile women.

Criteria for inclusion in the research were age from 25 to 40, verified CE (morphologically and immunohistochemically (CD 138+)), TPI, infertility on the EGE background, and the woman's informed consent for participation in research.

The examination of women included an assessment of complaints, anamnesis, general and gynecological examination, and standard laboratory examination (clinical and biochemical blood analysis, general urine analysis, and hemostasiogram).

With sonographic signs of CE, endometrial polyp (PE), and endometrial hyperplasia, hysteroscopy with biopsy sampling for morphological examination was performed on Days 7-9 of the menstrual cycle).

In the phase of the "implantation window" (on Days 20-22 of the menstrual cycle), 6-8 days after the peak of ovulation), aspiration Pipelle biopsy of the endometrium was performed.

Immunohistochemical data were crucial for distinguishing molecular phenotypes of the endometrium. Pathomorphological and immunohistochemical examination of the endometrium was performed according to the standard procedure. The obtained biopsies were fixed with a 10% buffered formalin solution for 24 hours, followed by standard histological wiring and paraffin-embedding procedures. Histological sections with a thickness of 4 microns were made using Sakura rotary microscopes and stained with hematoxylin and eosin. The study of the preparations was carried out using a light microscope with an increase from ×50 to ×1000.

Immunohistochemical examination of the endometrium was performed in the "implantation window" phase (luteinizing hormone peak (+ 7)) to assess the expression of cytokines, chemokines, growth factors: TNF-α, IL10, GM-CSF, and CXCL16 in the epithelium of the glands and stroma, BCA1 in the glandular epithelium, and TGF-β in the stroma. The analysis of the results was carried out considering the number of stained cells and the intensity of their staining. H-score was calculated according to the formula: H-score = $\Sigma(Pi \times i)$, where *Pi* is the percentage of stained cells for each intensity (from 0% to 100%), *i* is the intensity of staining with a value of 0 (no evidence of staining), 1 (weak staining), 2 (moderate staining), and 3 (strong staining). This score, therefore, is in the range of 0 to 300. The analysis of the results of the study with antibodies to TGF-β1 was performed only in the endometrial stroma by a semi-quantitative method by assessing the number of positive cells, regardless of the intensity of staining. Data was interpreted as follows: 0 (no positive stromal cells), 1+ (cell count of ≤ 24%), 2+ (cell count from 25% to 49%), and 3+ (cell count of ≥ 50%). The preparations were studied using a Leica DMLB light microscope with a standard set of optics.

The proliferative activity of the endometrium was assessed based on the expression of Ki-67 nuclear protein in epithelial cells and stroma.

The material was taken from the uterine cavity for microbiological examination with a double-cavity catheter for embryo transfer after the cervix was treated with a swab soaked in chlorhexidine solution. The exclusion of contamination of the material by microorganisms from the vagina and cervical canal was achieved by extending the inner part of the catheter into the uterine cavity, after which it was immersed in the outer part, then the system was removed. Endometrial samples were examined by RT-PCR (Femoflor 16 tests, NPO 50 DNA Technology LLC (Russia)) to assess the content of lactobacilli, opportunistic pathogens (*Mycoplasma hominis* and *Ureaplasma urealyticum* + *Ureaplasma parvum*) and pathogenic microorganisms (*Mycoplasma genitalium*) in genome-equivalent units (GE/ml) on the IQ5 Multicolor Real-Time PCR Detection System of BIO-RAD (USA). The microbial load was considered positive

when microorganisms in the samples were detected in an amount of more than 10^3 GE/ml.

The study was carried out in accordance with the Helsinki Declaration of the World Medical Association and approved by the Ethics Committee of the Medical Institute at the Peoples' Friendship University of Russia (RUDN University).

Statistical analysis was performed using the statistical software package SPSS version 22.0 (SPSS Inc, Armonk, NY: IBM Corp). The normality of the distribution of continuous variables was tested by the Shapiro-Wilk test. The results are presented as median (Me) and interquartile range (IQR [Q1; Q3]). The Mann-Whitney U test and Kruskal-Wallis test were used, respectively, to compare differences between 2 and 3 or more independent groups. Group comparisons with respect to categorical variables were performed using Pearson's chi-squared ($\chi 2$) test with Yates correction or, alternatively, Fisher's exact test when expected cell counts were less than 5. A probability value of $P<0.05$ was considered statistically significant.

Results and Discussion

The average age of women with infertility in the groups did not significantly differ: 31.6 (25.2;32.8) years in Group UI, 33.6 (28.2; 37.2) years in Group CE, 32.8 (27.6;36.4) years in Group TPI, 33.4 (29.2;37.2) years in Group EGE, and 31.2 (26.2; 35.4) years in Group TE. The average age of women in the comparison group was 30.6 (28.5; 36.6) years.

The basis for identifying endometrial phenotypes was immunohistochemical studies, considering the data of hysteroscopy and pathomorphological examination. Molecular signatures of the endometrium were detected: the impaired immune status phenotype (n=10) and dysplastic phenotype (n=11) in Group UI; the proliferative phenotype (n=12), chronic inflammation phenotype (n=20), "normal" phenotype (n=12) (indicators are identical to those in the comparison group), dysplastic phenotype (n=18), and impaired immune status phenotype (n=12) in Group TPI; the impaired immune status phenotype (n=10) and proliferative phenotype (n=12) in Group EGE; the chronic inflammation phenotype (n=10), proliferative phenotype (n=8), proliferative phenotype in combination with endometrial polyp (EP) (n=8), and dysplastic phenotype (n=10) in Group CE; and dysplastic phenotype (n=8) in Group TE. The indicators of healthy fertile women (the comparison group, n=10) were chosen as the reference.

Grouping of the selected variants showed that the basis of the "uterine factor" of infertility were the following phenotypes: impaired immune status phenotype, proliferative phenotype, dysplastic phenotype, chronic inflammation phenotype, and "normal" endometrium phenotype. The expression profile of markers in which is presented in Figures 1-10.

Women with a "normal" endometrial phenotype were distinguished by the balanced secretion of cytokines with moderate activation of the inflammatory molecular network modulated by stroma cells to control implantation, migration and invasion of the trophoblast. The proinflammatory Th1-immune response is considered as the activity of biologically active substances necessary for blastocyst implantation.[8] The leading role in controlling the network of immunoregulatory molecules is associated with TNF-α overexpression, the development of a local inflammatory response, and the induction of tolerant properties of dendritic cells.[9]

We believe that the predominance of the LDM type in the endometrium should be considered from the standpoint of participation in limiting the inflammatory "immune response" and activating the mechanism of immunological tolerance in the presence of Treg-dominant in the Treg/Th17 ratio.[10] These immune-microbial interactions serve as a criterion for the likelihood of blastocyst implantation. Microbial homeostasis in the endometrium might form not only the resistance to colonization by opportunistic flora, but also the ability to express genes that affect the adequate level of receptors for sex steroid hormones in the "implantation window" phase.[11]

The morphological basis of the dysplastic phenotype of the endometrium was dystrophic-atrophic changes. Immunoregulation disorders in the dysplastic phenotype consisted of a "poor immune response" combined with depletion or blocking of energy substrates. Disorders in the adequate preparation of the endometrium during the "implantation window" are due to a marked decrease in expression, in contrast to the comparison group, both in the glandular compartment of the endometrium (GM-CSF – 3.7 times, TNF-α – twice, IL-10 – 1.8 times, CXCL16 – twice, BCA1 – 2.6 times) and in the stroma (GM-CSF – 2.7 times, TNF-α – 4.6 times, IL-10 – 2.9 times, CXCL16 – 3.3 times, with a maximum TGF-β1). It is reported that the cause of implantation failures may be premature "aging" of the endometrium due to immune "stresses" and inflammatory damage.[12] TGF-β1 overexpression with activation of the nuclear factor signaling pathway NF-kB, TGF-β1/Smad3/Smad7 is believed to be one of the reasons for the formation of intrauterine synechiae.[13] Disorders of cellular metabolism in the dysplastic phenotype in the presence of NLDM type (47.1%) are likely to be revealed as a consequence of exposure to toxic metabolites caused by the persistence of low-immunogenic infections.

The disorders in the histological dating of the endometrium in 21.9% of women with a phenotype of impaired immune status indicate the criticality of taking into account the markers of "maturity," especially in IVF/IVF-ICSI protocols for synchronization with the developing embryo. The imbalances in the endometrial cytokine cascades and absence of proinflammatory Th1 shift during the implantation window (the expression of GM-CSF, BCA1, CXCL16 decreased by 1.4 times, TNF-α – by 1.3 times, IL-10 – by 1.2 times in the glandular epithelium; in the stroma, the expression of GM-CSF, TNF-α, CXCL16 decreased by 1.3 times, IL-10 – by 1.2 times) disrupt the ability of stroma cells to decidualize. Implantation failures are associated with the impairment in immune tolerance caused by a decrease in the number and function of Treg cells.[14]

The phenotype of chronic inflammation was determined by the excess expression of proinflammatory cytokines in the epithelium of the endometrial glands in comparison with anti-inflammatory ones (an increase in TNF–α by 1.1 times, GM-CSF, CXCL16, BCA1 by 1.2 times, a decrease in IL-10 by 2 times). In the endometrial stroma, the expression level of GM-CSF, TNF-α, CXCL16 was significantly higher than in the control by 1.2 times, and IL-10 was lower by 1.8 times, and the level of TGF-β was the lowest in comparison with other groups.

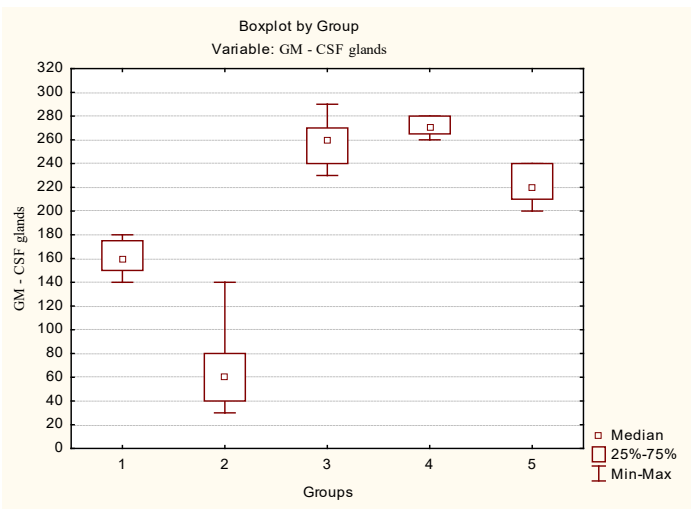

Fig. 1. *Expression of GM-CSF in glandular epithelium.*

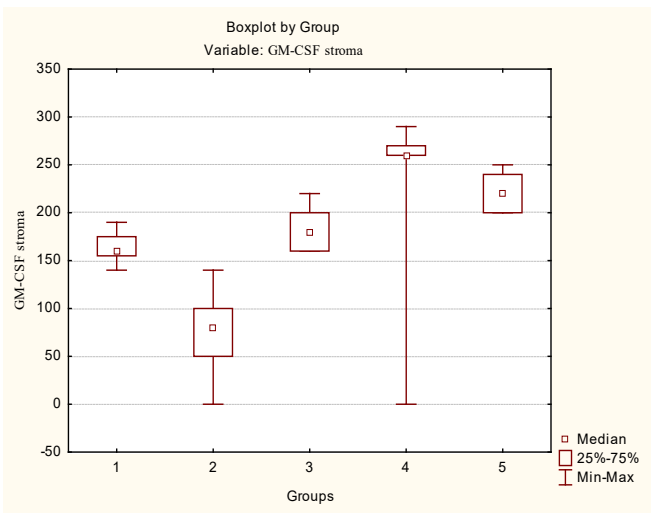

Fig. 2. *Expression of GM-CSF in endometrial stroma.*

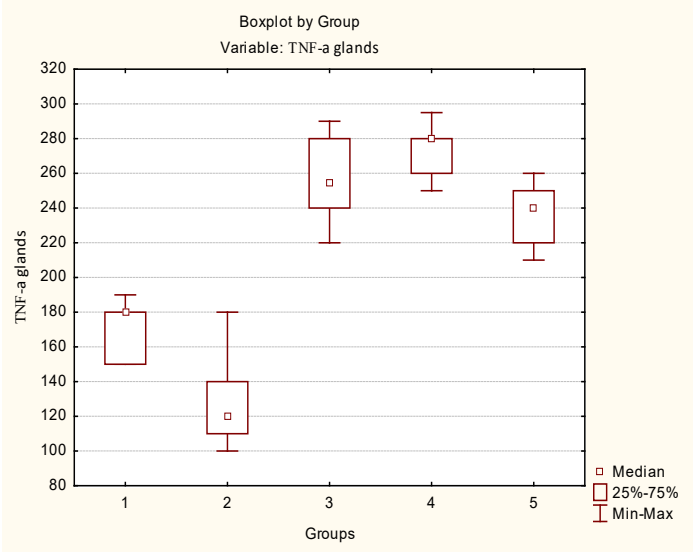

Fig. 3. *Expression of TNF-α in glandular epithelium.*

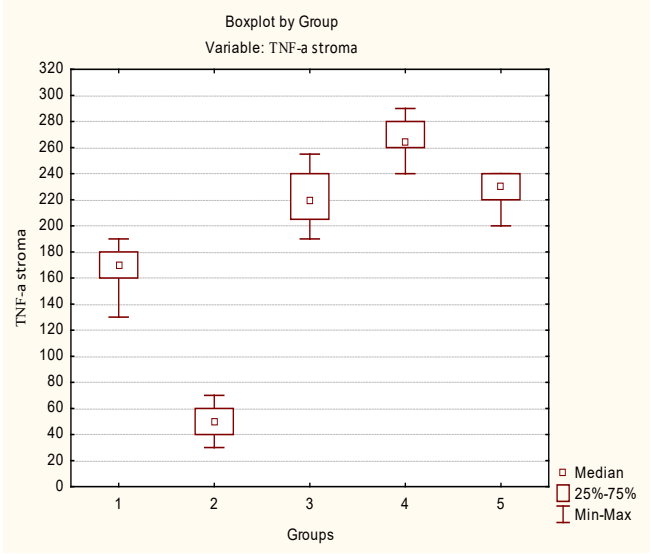

Fig. 4. *Expression of TNF-α in endometrial stroma.*

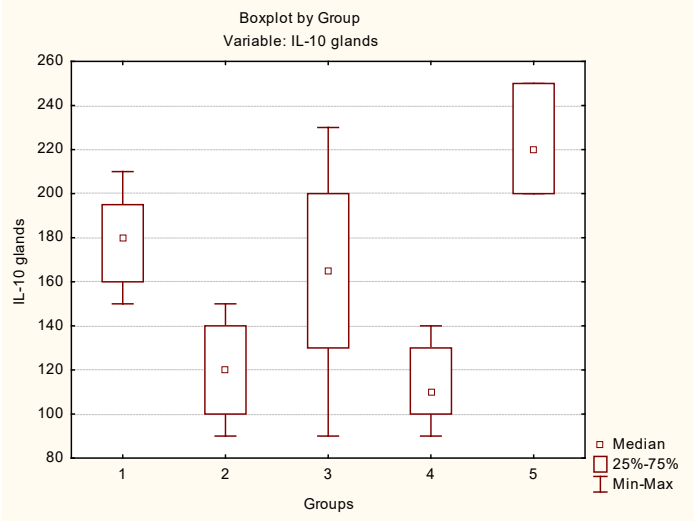

Fig. 5. *Expression of IL-10 in glandular epithelium.*

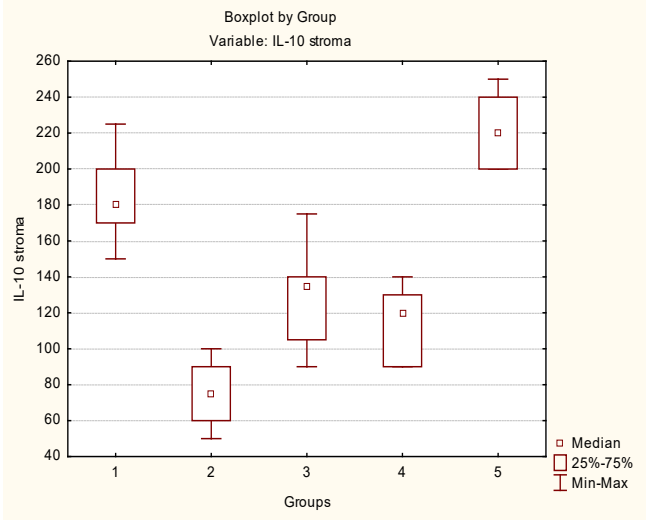

Fig. 6. *Expression of IL-10 in endometrial stroma.*

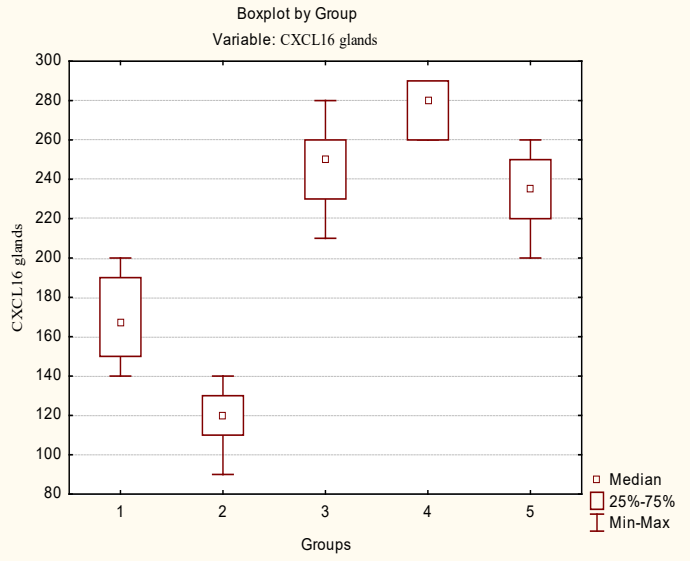

Fig. 7. *Expression of CXCL16 in glandular epithelium.*

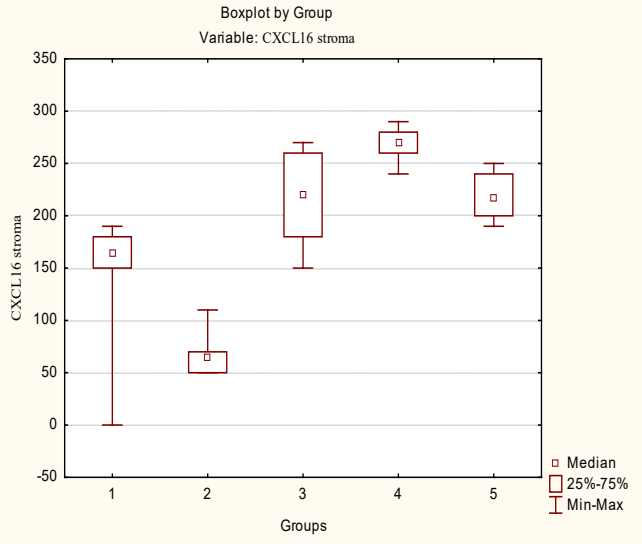

Fig. 8. *Expression of CXCL16 in endometrial stroma.*

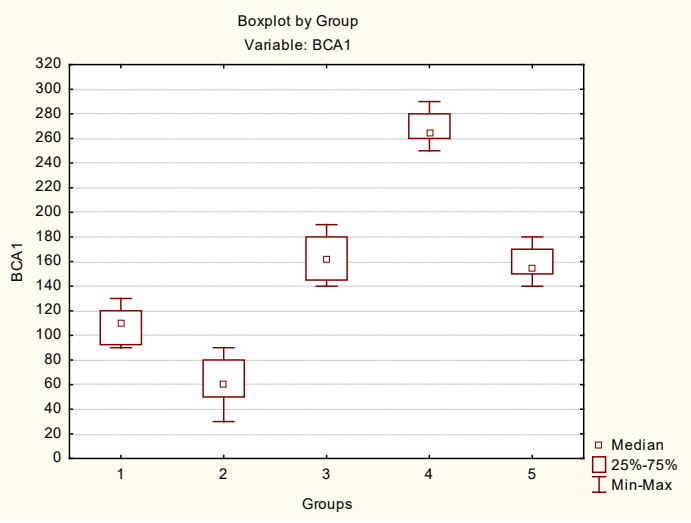

Fig. 9. *Expression of BCA1 in glandular epithelium.*

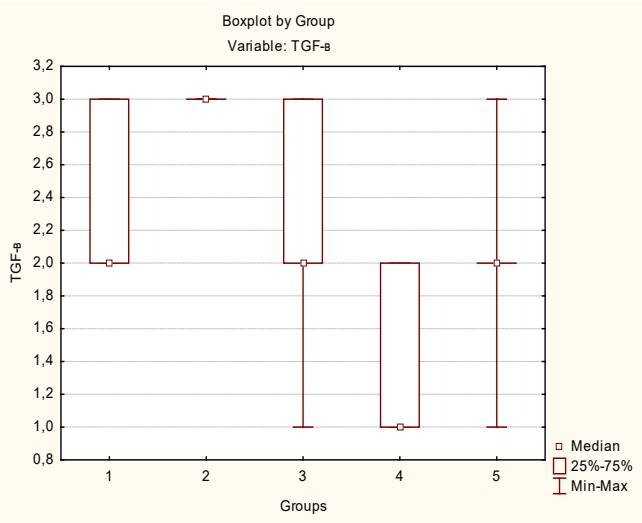

Fig. 10. *Expression of TGF-β in endometrial stroma.*

Figures 1-10. *Groups: 1 – impaired immune status phenotype, 2 – dysplastic phenotype, 3 – proliferative phenotype, 4 – chronic inflammation phenotype, 5 – "normal" endometrium phenotype*

The abnormal immune microenvironment of the "inflamed" endometrium is associated with the introduction of pathogenic bacteria, the main component of whose membrane (lipopolysaccharide) mediates an increase in the level of chemokine CXCL13 (BCA1), inflammation, and recruitment into the stroma of the B cell pool.[15] The infiltration of the stroma by plasma cells leads to changes in the architectonics and dysfunction of the endometrium. A decrease in the expression of TGF-β and IL-10 in CE is believed to be the cause of a quantitative or functional deficiency of the anti-inflammatory clone of Treg cells on the background of an inflammatory stroma reaction, fibrosis, and implantation failures.[16]

The dysbiotic profile of the endometrium in 47.6% of infertile women with CE appears to cause unfavorable molecular mechanisms for implantation. The impairment in microbial homeostasis in CE (the prevalence of a NLDM type in 63.3%, as well as the excessive growth of *Gardnerella vaginalis*, *Ureaplasma* spp. and mixes of *Atopobium vaginae*/*Enterobacteriaceae* in the absence of a lactobacilli bacteria) probably stimulates the overexpression of proinflammatory cytokines that create a microenvironment aggressive for blastocyst implantation. We believe that the revealed heterogeneity of the composition of the uterine microbiome in women with CE explains the heterogeneity of data on reproductive outcomes – from infertility, recurrent implantation failures and miscarriages to successful delivery. Our data complement the ideas about the breakdown of the mechanisms of adaptation to the constant exposure to microbes in CE and, as a result, the inadequacy of the immune response and the "vicious circle" of chronic inflammation.

The molecular profile of women with a proliferative endometrial phenotype was characterized by the immunomodulatory activity of GM-CSF (increased expression

by 1.2 times) on the background of moderate overexpression of TNF-α, CXCL16 (1.1 times) and a decrease in IL-10 (1.3 times) in the glandular epithelium. In the endometrial stroma, significant differences from the comparison group were revealed only in relation to GM-CSF – by 1.2 times and IL-10 – by 1.6 times**.** The imbalance in local cytokine production confirms the participation in the formation of a proliferative phenotype of the endometrium not only of proinflammatory cytokines (GM-CSF, TNF-α, CXCL16), the overexpression of which is associated with an increase in cytotoxic T cells, but also impairments in local steroidogenesis.

With a proliferative endometrial phenotype, disorders of the uterine microbiome were less common than with chronic inflammation (NLDM type - 31.6%). A decrease in lactobacilli level with a relative increase in the levels of *Firmicutes*, *Proteobacteria*, *Actinobacteria*, *Fusobacteria*, *Bacteroides*, *E. coli,* and *Bacteroides fragilis* was observed in hyperplastic processes of the endometrium.[17] The participation of microbiota and immune-inflammatory "networks" in the genesis of focal endometrial hyperplasia is proposed to be considered through an increase in the activity of the β-glucuronidase enzyme in the presence of certain bacteria, followed by an increase in the level of local estrogens.

Conclusion

CE was verified in 57.1% of women. In the regulation of complex mechanisms of implantation, the participation of molecular interactions of microbiota (LDM and NLDM types) and immunocompetent mediators (cytokines, chemokines, growth factors), predictive of impaired decidual transformation and expression of genes involved in the regulation of endometrial receptivity, has been revealed.

Competing Interests

The authors declare that they have no competing interests

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International Journal of Biomedicine 13(4) (2023) 261-268
http://dx.doi.org/10.21103/Article13(4)_OA5

ORIGINAL ARTICLE

Obstetrics and Gynecology

INTERNATIONAL JOURNAL OF BIOMEDICINE

Cardiometabolic Risk Factors and Its Association with Hyperandrogenemia Among Sudanese Reproductive Women with Polycystic Ovary Syndrome

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Abstract

Background: Risk factors for cardiovascular disease (CVD) are more common and frequently occur among PCOS women. The objective of this study was to evaluate atherogenic index of plasma (AIP) as a predictor of CVD and its association with hyperandrogenemia among PCOS women.

***Methods and Results*:** This hospital-based study, conducted in Khartoum (Sudan) from October 2020 to September 2021, used a case-control design. The patients (n=150) were women with diagnosed PCOS, according to Rotterdam criteria. The controls were 150 infertile women who did not have PCOS. An ELISA reader (ASYS Expert Plus Microplate, Austria) was used to quantify serum insulin, luteinizing hormone (LH), follicle-stimulating hormone (FSH), and anti-Mullerian hormone (AMH) by indirect methods and total testosterone (TT) by competitive method during the follicular phase of the menstrual cycle. Serum samples of total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and fasting plasma glucose (FPG) were assayed on the Cobas c311 system (Roche Diagnostics GmbH, Germany). The triglyceride–glucose index (TyGI) and TyGI-BMI, as a marker for insulin resistance (IR), were calculated. The logarithmically converted ratio of the molar concentrations of TG to HDL-C was used to determine the AIP. Low CVD risk was defined as < 0.1, medium risk as 0.1– 0.24 and high risk as >0.24.

PCOS women had a significant increase in BMI, compared to non-PCOS (P<0.05). Moreover, 73.4% of PCOS women were overweight to obese. PCOS women were found to have significantly increased serum levels of TC, TG, and LDL-C, as well as significantly increased levels of HOMA-IR and AIP, and a significantly decreased level of HDL-C, compared with non-PCOS women. Overall, among PCOS women, 30.0% had high TC (≥200 mg/dL), 24.7% - high TG (≥150 mg/dL), 29.3% - high LDL-C (≥130 mg/dL), and 46.7% - lower HDL-C (<40 mg/dL). Moreover, 40.6% of PCOS women had medium-to-high CVD risk, and their mean AIP was >0.1. PCOS women with hyperandrogenemia showed significantly increased AIP and decreased HDL-C. Additionally, about 73% of PCOS women with hyperandrogenemia had lower HDL-C, and 29.9% had a high risk of CVD (AIP>0.24). A Spearman correlation revealed that PCOS women's TT correlates positively with TC, TG, TyGI, and AIP and inversely correlates with HDL-C. AIP positively correlates with TT, TC, TyGI, and TyGI-BMI index.

Conclusion: Our data revealed a significant occurrence of hyperandrogenemia, dyslipidemia, AIP, and obesity, all of which are considered risk factors for CVD in PCOS women. PCOS women should be screened, diagnosed, and treated early, which will almost certainly reduce the overall burden of CVD. **(International Journal of Biomedicine. 2023;13(4):261-268.)**

Keywords: PCOS • hyperandrogenemia • hyperlipidemia • atherogenic index of plasma

For citation: Elmugadam A, Alhassan SM, Elfadil GA, Altoum A, Ismail MK, Ahmed SM. Cardiometabolic Risk Factors and Its Association with Hyperandrogenemia Among Sudanese Reproductive Women with Polycystic Ovary Syndrome. International Journal of Biomedicine. 2023;13(4):261-268. doi:10.21103/Article13(4)_OA5

Abbreviations

AIP, atherogenic index of plasma; **AMH**, anti-Mullerian hormone; **BMI**, body mass index; **CVD**, cardiovascular disease; **FPG**, fasting plasma glucose; **FSH**, follicle-stimulating hormone; **HDL-C**, high-density lipoprotein cholesterol; **IR**, insulin resistance; **LH**, luteinizing hormone; **LDL-C**, low-density lipoprotein cholesterol; **PCOS**, polycystic ovarian syndrome; **TT**, total testosterone; **TC**, total cholesterol; TG, triglycerides; **TyGI**, triglyceride–glucose index; **T2DM**, type 2 diabetes mellitus.

Introduction

Cardiovascular disease (CVD) is the main cause of death in women worldwide.[1] Risk factors for CVD are more common and frequently occur among PCOS women.[2,3] It is the most prevalent endocrine illness in women of reproductive age.[4] Cardiometabolic abnormalities, such as obesity, dyslipidemia, T2DM, hypertension, metabolic syndrome, and low-grade inflammation, have been linked to PCOS and have been shown to enhance the risk for CVD.[5-7]

Atherogenic hyperlipidemia, the joint occurrence of high fasting TG levels and low levels of HDL-C, is common among patients with metabolic disorders.[8,9] Single lipid indicators are thought to be less accurate predictors of CVD than comprehensive lipid ratios.[10] In this regard, Log10 of the ratio of the molar concentration of TG to HDL-C is used to establish the atherogenic index of plasma (AIP).[10] It possesses a solid and independent prognostic factor for CVD and has demonstrated a good association with LDL-C.[11]

Additionally, atherosclerosis and myocardial infarction have been strongly predicted by AIP.[12,13] TG/HDL-C ratio and fasting triglyceride–glucose index (TyGI) have been linked to CVD in previous research.[14,15] Moreover, some studies have shown that the TC/HDL-C ratio correlates with IR and CVD risk.[16,17]

Investigating CVD risk among PCOS women is important for the investigators as well as the treating doctors. There is no published information on CVD risk among PCOS in Sub-Saharan Africa, including Sudan. Contradictory views on the pattern of atherogenic lipid profile and anthropometric measurement in PCOS encouraged us to conduct the present study.

The objective of this study was to evaluate AIP as a predictor of CVD and its association with hyperandrogenemia among PCOS women.

Materials and Methods

This hospital-based study, conducted in Khartoum (Sudan) from October 2020 to September 2021, used a case-control design. The patients (n=150) were women with diagnosed PCOS, according to Rotterdam criteria. The controls were 150 infertile women who did not have PCOS.

Exclusion criteria were women suffering from CVD and diabetes, women using oral contraceptives, glucocorticoids, ovulation-inducing drugs, and estrogen and anti-androgen medications.

There were no published statistics on the prevalence of PCOS in Sudan when the study was conducted. The prevalence of PCOS in unspecified populations is 3%–10%.

The Sudanese Federal Ministry of Health in Khartoum approved the study protocol. All study participants provided thorough sociodemographic details and medical and gynecological history, including information on menstrual patterns, fertility, and hirsutism. Then, comprehensive general and pelvic exams were carried out.

Weight was measured twice using customary procedures. OMRON BF508l Body Fat Scales (China) were used following calibration. Weight was calculated with a precision of 0.1kg. After calibration, height was measured twice using a portable stadiometer (SECA-213 model, Germany). BMI was calculated and categorized as underweight (<18.5 kg/m^2), normal (18.5-24.9 kg/m^2), overweight (25.0-29.9 kg/m^2), and obese (≥30 kg/m^2), based on WHO classification.[18]

Women were asked to return on days 2 through 5 following an unforeseen period or on a convenient day if they experienced amenorrhea. Between the hours of 8 and 9 in the morning, 5mL of venous blood was drawn. Hettich Centrifuge D-78532 (Tuttlingen, Germany) was used to centrifuge the blood, and the plasma was then collected and stored at -20°C until the test. An ELISA reader (ASYS Expert Plus Microplate, Austria) was used to quantify serum insulin, LH, FSH, and AMH by indirect methods and TT by competitive method during the follicular phase of the menstrual cycle. Serum samples of TC, TG, LDL-C, HDL-C, and FPG were assayed on the Cobas c311 system (Roche Diagnostics GmbH, Germany). Every time the methodologies employed in this investigation were tested for precision and accuracy, commercially prepared control sera were added to each batch for analysis. The logarithmically converted ratio of the molar concentrations of TG to HDL-C was used to determine the AIP. Low CVD risk was defined as <0.1, medium risk as 0.1– 0.24 and high risk as >0.24.[13] The TyGI and TyGI-BMI, as a marker for IR, were calculated. The TyGI was calculated using the following formula: ln (fasting TG [mg/dL]×FPG [mg/dL]/2).[19] TyGI-BMI, as a marker for IR, was calculated as ln [TG (mg/dl) × FBG (mg/dl)/2] × BMI (kg/m^2).[20] TT>109.5 ng/dL was the threshold for hyperandrogenism.[21]

Statistical analysis was performed using the statistical software package SPSS version 26.0 (SPSS Inc, Armonk, NY: IBM Corp). The normality of the distribution of continuous variables was tested by the one-sample Kolmogorov-Smirnov test. Continuous variables with normal distribution were presented as mean (standard deviation [SD]); non-normal variables were reported as median (Me) and interquartile range (IQR [Q1;Q3]). For data with normal distribution, inter-group comparisons were performed using Student's t-test. The Mann-Whitney U Test was used to compare the differences between the two independent groups (for nonparametric data). Group comparisons with respect to categorical variables are performed using chi-square test. Spearman's rank correlation coefficient (R) was calculated to measure the strength and direction of the relationship between two variables. Multiple linear regression was applied using several explanatory

variables to predict the outcome of a dependent variable (AIP). A probability value of $P<0.05$ was considered statistically significant.

Results

PCOS women had a significant increase in BMI, compared to non-PCOS ($P<0.05$). Moreover, 73.4% of PCOS women were overweight to obese. In addition, PCOS women revealed a significant increase in LH, LH/FSH ratio, AMH, and TT levels, and a significant decrease in FSH, compared to non-PCOS women; 70.0% of PCOS women had increased LH/FSH ratio (>1), and 58.7% had hyperandrogenemia (TT>109.5 ng/dL). About 52% of PCOS women had menstrual cycle irregularity (Table 1).

Table 1.
Baseline data of PCOS women and non-PCOS women.

Variable	PCOS (n = 150)	Non-PCOS (n = 150)	*P*- value
Age, year			
Mean ± SD	29.03 ± 6.0	28.5 ± 5.5	0.683
Me [Q1-Q3]	28.5 [25.0- 33.0]	28.5 [24.0- 32.0]	
BMI, kg/m^2			
Mean ± SD	28.6 ± 4.8	22.9 ± 1.3	0.000
Me [Q1-Q3]	28.4 [24.7- 31.6]	22.8 [22.2- 24.0]	
BMI classification			
Normal, n (%)	40 (26.7)	146 (97.3)	0.000
Overweight, n (%)	61 (40.7)	4 (2.7)	
Obese, n (%)	49 (32.7)	0 (0)	
LH, mIU/mL			
Mean ± SD	10.5 ± 6.2	4.4 ± 1.4	0.000
Me [Q1-Q3]	9.2 [5.6 - 14.7]	4.2 [3.3 - 5.2]	
FSH, mIU/mL			
Mean ± SD	6.6 ± 2.9	8.2 ± 2.2	0.000
Me [Q1-Q3]	6.4 [4.9 - 8.2]	8.1 [6.3 - 9.8]	
LH:FSH ratio			
Mean ± SD	1.88 ± 1.4	0.55 ± 0.12	0.000
Me [Q1-Q3]	1.39 [0.91 - 2.27]	0.55 [0.47 - 0.64]	
LH:FSH ratio			
≥ 1, n (%)	45 (30.0)	150 (100.0)	0.000
>1, n (%)	105 (70.0)	0 (0.0)	
TT, ng/dL			
Mean ± SD	214.1 ± 167.2	44.1 ± 28.4	0.000
Me [Q1-Q3]	163.0 [64.7 - 390.0]	39.4 [18.9 - 67.3]	
TT level			
≤109.5 (ng/dL), n (%)	62 (41.3)	148 (98.7)	0.000
>109.5 (ng/dL), n (%)	88 (58.7)	2 (1.3)	
AMH, ng/mL			
Mean ± SD	7.2 ± 3.3	2.3 ± 1.6	0.000
Me [Q1-Q3]	6.1 [5.0 - 7.72]	2.4 [1.6 – 3.0]	

Furthermore, PCOS women were found to have significantly increased serum levels of TC, TG, and LDL-C, as well as significantly increased levels of TyGI-BMI index, insulin, HOMA-IR, and AIP, and a significantly decreased level of HDL-C, compared with non-PCOS women. However, PCOS women revealed insignificant differences in TyGI and FPG when compared with non-PCOS women. Overall, among PCOS women, 30.0% had high TC (≥200 mg/dL), 24.7% - high TG (≥150 mg/dL), 29.3% - high LDL-C (≥130 mg/dL), and 46.7% - lower HDL-C (<40 mg/dL) (Table 2). Moreover, 40.6% of PCOS women had medium-to-high CVD risk, and their mean AIP was >0.1 (Table 2).

Table 2.

Classical and non-classical cardiometabolic risk factors among reproductive women with and without PCOS.

Variable	PCOS (n=150)	Non-PCOS (n=150	*P*-value
TC, mg/dL			
Mean ± SD	190.3 ± 36.8	148.4 ± 22.0	0.000
Me [Q1-Q3]	186.5 [163.7 - 222.0]	151.5[127.0 - 167.0]	
TC level			
≤200 (mg/dL), n (%)	96 (64.0)	149 (99.3)	0.000
>200 (mg/dL), n (%)	54 (30.0)	1.0 (0.67)	
TG, mg/dL			
Mean ± SD	119.6 ± 53.9	108.1 ± 38.1	0.034
Me [Q1-Q3]	105.0 [83.0 - 150.5]	99.0 [78.5 - 136.0]	
TG level			
≤150 (mg/dL), n (%)	113 (75.3)	121 (80.7)	0.265
>150 (mg/dL), n (%)	37 (24.7)	29 (19.3)	
LDL-C, mg/dL			
Mean ± SD	113.9 ± 30.0	84.7 ± 25.3	0.000
Me [Q1-Q3]	113.5 [92.2 - 135.0]	86.5 [66.0 - 104.2]	
LDL-C level			
≤130 (mg/dL), n (%)	106 (70.7)	147 (98.0)	0.000
>130 (mg/dL), n (%)	44 (29.3)	3 (2.0)	
HDL-C, mg/dL			
Mean ± SD	39.9 ± 4.7	47.4 ± 9.1	0.000
Me [Q1-Q3]	40.0 [37.0 - 43.0]	45.0 [40.0 - 53.0]	
HDL-C classification			
<40(mg/dL), n (%)	70 (46.7)	22 (14.7)	0.000
≥40 (mg/dL), n (%)	80 (53.3)	128 (85.3)	
FPG, mg/dL			
Mean ± SD	89.9 ± 12.5	89.7 ± 12.6	0.916
Me [Q1-Q3]	89.0 [81.5 - 97.0]	90.0 [79.0 - 96.2]	
Insulin, mIU/ml			
Mean ± SD	4.48 ± 3.6	0.71 ± 0.7	0.000
Me [Q1-Q3]	2.2 [1.2 - 8.7]	0.5 [0.3 - 0.8]	
HOMA-IR			
Mean ± SD	0.98 ± 0.7	0.15 ± 0.1	0.000
Me [Q1-Q3]	0.45 [0.27 - 1.74]	0.11 [0.06 - 0.17]	

Table 2 (continued).

Classical and non-classical cardiometabolic risk factors among reproductive women with and without PCOS.

Variable	PCOS (n=150)	Non-PCOS (n=150	*P*-value
TyGI			
Mean ± SD	4.5 ± 0.2	4.5 ± 0.2	1.000
Me [Q1-Q3]	4.56 [4.44 - 4.73]	4.53 [4.39 - 4.72]	
TyGI–BMI index			
Mean ± SD	131.8 ± 23.1	104.2 ± 7.3	0.000
Me [Q1-Q3]	130.7 [114.1 - 146.5]	104.4 [99.6 - 108.8]	
AIP			
Mean ± SD	0.08 ± 0.1	-0.022 ± 0.1	0.000
Me [Q1-Q3]	0.017 [-0.09 - 0.18]	-0.023 [-0.15 - 0.12]	
AIP classification			
Low risk, n (%)	89 (59.3)	109 (72.2)	0.000
Medium risk, n (%)	26 (17.3)	30 (20.0)	
High risk, n (%)	35 (23.3)	11 (7.3)	

PCOS women with hyperandrogenemia showed significantly increased AIP and decreased HDL-C, but insignificant differences in age, BMI, TC, TG, LDL-C, TyGI, TyGI-BMI index, and HOMA-IR (Table 3). Additionally, about 73% of PCOS women with hyperandrogenemia had lower HDL-C (<40 mg/dL), and 29.9% had a high risk of CVD (AIP>0.24) (Figure 1).

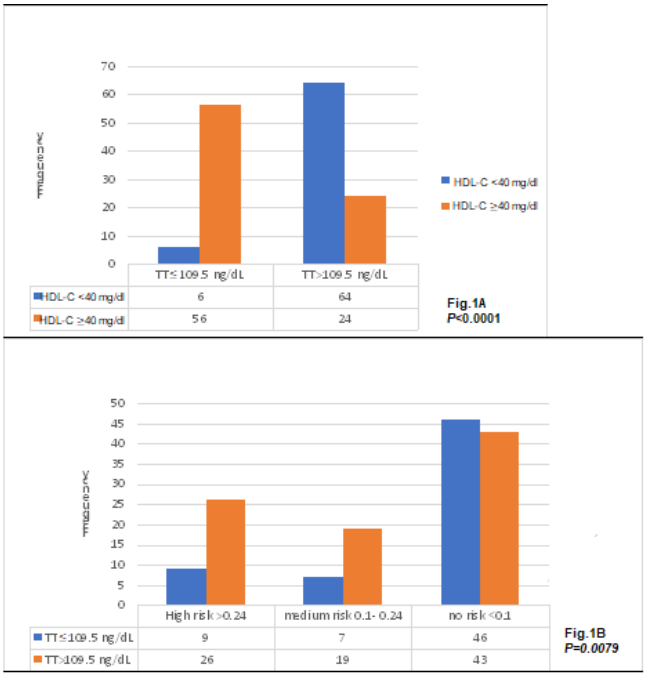

Figure 1.

(A) HDL-C level in association with TT level.

(B) Risk of CVD based on AIP in association with TT level.

Table 3.

Classical and non-classical cardiometabolic risk factors among reproductive women with PCOS based on hyperandrogenemia (cut-off > 109.5 ng/dL)

Variable	TT, ng/dL Mean ± SD		P-value
	≤ 109.5 (n= 62)	>109.5 (n= 88)	
Age, year	29.7 ± 4.9	28.4 ± 5.7	0.202
BMI, kg/m^2	29.0 ± 4.9	28.4 ± 4.7	0.431
TC, mg/dL	185.5 ± 35.5	193.7 ± 37.6	0.178
TG, mg/dl	110.0 ± 45.5	126.5 ± 58.5	0.067
LDL-C, mg/dl	111.5 ± 29.5	115.5 ± 30.4	0.413
HDL-C, mg/dl	43.3 ± 3.8	37.5 ± 3.5	0.000
TyGI	4.5 ± 0.1	4.6 ± 0.2	0.163
TyGI–BMI index	132.7 ± 22.8	131.3 ± 23.8	0.717
FPG, mg/dL	91.7 ± 3.7	88.6 ± 11.5	0.159
Insulin, mIU/ml	4.6 ± 3.7	4.3 ± 3.5	0.706
HOMA-IR	1.17 ± 0.5	1.24 ± 0.6	0.488
AIP	0.01 ± 0.1	0.13 ± 0.1	0.000

A Spearman correlation revealed that PCOS women's TT correlates positively with TC, TG, TyGI, and AIP and inversely correlates with HDL-C. AIP positively correlates with TT, TC, TyGI, and TyGI-BMI index (Table 4).

The multiple linear regression model determined the factors affecting the AIP level among PCOS women. The dependent variable entered in the model was AIP level, whereas age, BMI, LDL-C, TT, and TyGI were the independent variables. Findings revealed that AIP increased significantly with TT, LDL-C, and TyGI (Table 5).

Discussion

Some cardiometabolic risk factors were examined in the current study among Sudanese PCOS. It is thought that obesity, hyperlipidemia, IR, and hyperandrogenemia may be the root causes of this risk. PCOS women's BMI was noticeably greater than non-PCOS. Additionally, 32.7% of PCOS women were obese. Mohammed et al.[22] demonstrated that BMI was significantly higher among Sudanese women with PCOS. Obesity in 31.4% of cases was reported among infertile Jordanian women with PCOS.[23] A higher frequency (63.7%) of obesity was noted in PCOS women in California.[24] This difference may depend on race, ethnicity, location, and environmental factors.

In this study, we hypothesized that PCOS women would suffer from dyslipidemia/hyperlipidemia and AIP. The results of our study showed that low HDL-C was the most common type of dyslipidemia, which was in line with other studies' findings.[25,26]

Table 4.

Spearman correlation between serum TT, AIP and cardiometabolic risk factors among PCOS women.

TT										
Variables	Age	BMI	TC	TG	LDL-C	HDL-C	TyGI	TyGI-BMI	HOMA-IR	AIP
R	-0.103	-0.042	0.184	0.215	0.120	-0.647	0.166	0.012	-0.043	0.345
P-value	0.211	0.608	0.024	0.008	0.144	0.000	0.043	0.882	0.606	0.000
AIP										
	Age	BMI	TC	TG	LDL-C	HDL-C	TyGI	TyGI-BMI	HOMA-IR	TT
R	-0.137	0.005	0.480	0.963	0.331	-0.536	0.898	0.236	0.028	0.345
P-value	0.095	0.953	0.000	0.000	0.000	0.000	0.000	0.004	0.733	0.000

Table 5.

Multiple linear regression with AIP level being a dependent variable.

	Unstandardized coefficients		Standardized coefficients			95% CI	
	B	Std. error	Beta	T	Sig.	Lower bound	Upper bound
Age, years	-0.001	0.001	-0.026	-0.858	0.392	-0.003	0.001
BMI, kg/m^2	-0.001	0.001	-0.036	-1.225	0.223	-0.004	0.001
LDL-C, mg/dL	0.000	0.000	0.076	2.473	0.015	0.000	0.001
TT, ng/dL	0.021	0.004	0.181	6.012	0.000	0.014	0.028
TyGI	0.816	0.029	0.868	28.514	0.000	0.760	0.873

Age, BMI, LDL-C and TyGI were the independent variables entered in the regression model.

The sum of squares = 5.721, R^2 = 0.877, R = 0.936, adjusted R^2 = 0.872, df^2 = 143, F change= 203.467, and significance=0.000.

Our research supported the findings of Iuhas et al.[27] about higher TC and LDL-C levels and lower HDL-C levels in PCOS women. Previous studies by Macut et al.[28] and Lath et al.[29] found that about 70% of women with PCOS had elevated lipid levels. PCOS women exhibited adverse lipid profiles, including a low level of HDL-C, high levels of TG, TC, and LDL-C, and significantly higher lipoprotein concentrations.[30-32] Different classes of dyslipidemia among women with PCOS may be because of androgenemia and IR, which are usually seen among PCOS women.

According to our findings, 58.7% of women with PCOS exhibited hyperandrogenemia (TT>109.5 ng/dL). Likewise, Livadas et al. reported that the prevalence of hyperandrogenemia was 58.8%,[33] but other studies indicated greater prevalence rates of 78.2% and 80%.[34,35] In women with PCOS, hyperandrogenism has been linked favorably to the degree of metabolic dysfunction.[36] Hyperandrogenemia is another risk factor for vascular diseases.[37] This study revealed significantly increased TT values in PCOS women, compared to non-PCOS women.

A direct relation between dyslipidemia and the risk of CVD is well known.[13,38] The AIP is regarded as a stand-alone measure for estimating cardiac risk.[39] Our study revealed a significant association between AIP and TC, LDL-C, TT, and TyGI. Several studies reported that PCOS raises the risk of clinical CVD events.[40,41] Additionally, PCOS women with hyperandrogenemia (TT>109.5 ng/dL) had a significant increase in the mean of AIP and decreased HDL-C level, compared with PCOS women with normal androgen. Moreover, we examined the dependence of AIP on age, BMI, TC, LDL-C, TT, and TyGI. Our study found that AIP was positively associated with TC, LDL-C, TT, and TyGI.

Our finding agreed with the study by Abashova et al.,[42] which demonstrated that PCOS women with hyperandrogenemia had a significant decrease in HDL-C level as an anti-atherogenic type of lipoproteins, compared with normo-androgenic PCOS. Thus, a decreased serum HDL-C level may be associated with cell system failures in implementing anti-inflammatory and antioxidant protection, which contributes to the development of atherogenic dyslipidemia in PCOS. Laura et al.[43] first suggested a positive association between the TyGI and CVD events, including coronary heart failure, cerebrovascular disease, and peripheral arterial disease, independent of confounding factors.

Increased serum cholesterol, LDL, triglycerides, and reduction in HDL indicate the presence of dyslipidemia. Some factors may contribute to the changes in lipid metabolism in patients with T2DM, including IR and/or relative insulin

deficiency and hyperglycemia. The marked significant reduction in vitamins A, E, C, and zinc has been reported by various studies, indicating metabolic abnormalities, which are related to increased cardiometabolic risks. Such reduction may be attributed to the increase in the need to control the excessive oxidative stress produced by abnormalities in glucose metabolism.[44]

The use of AIP may contribute to better and earlier identification of patients at high risk of CVD, especially among those with PCOS and hyperandrogenemia. Women with PCOS, in particular, frequently visit gynecologists for help with infertility and menstruation problems. PCOS patients exhibit a higher incidence of CVD risk factors than non-PCOS women. PCOS women should be screened, diagnosed, and treated early, which will almost certainly reduce the overall burden of CVD.

It is essential to consider the limitations of our study. In the future, a longitudinal study should be carried out to monitor these PCOS women for a certain amount of time and report the actual incidence of CVD. Our findings are regarded as the first study on Sudanese women with PCOS to provide information on the CVD risk. It focused on obesity, AIP, dyslipidemia, and hyperandrogenemia.

Conclusion

The risk of CVD in PCOS-afflicted Sudanese women was brought up in this study. Given that our data revealed a significant occurrence of hyperandrogenemia, dyslipidemia, AIP, and obesity, all of which are considered risk factors for CVD, more research is required on PCOS women under the age of 40. Additionally, relevant actions must be taken to ensure that the general population is educated about PCOS, CVD risk, and their prevention by a multidisciplinary team. The management of PCOS patients should include the early identification of CVD risk factors.

Ethical Considerations

The study was conducted in accordance with ethical principles of the Declaration of Helsinki and approved by the Sudanese Federal Ministry of Health (#: 10-2020). All participants provided written informed consent.

Acknowledgments

We express our gratitude to all our collaborators and workers who made this study possible.

Competing Interests

The authors declare that they have no competing interests.

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International Journal of Biomedicine 13(4) (2023) 269-276
http://dx.doi.org/10.21103/Article13(4)_OA6

ORIGINAL ARTICLE

Obstetrics and Gynecology

INTERNATIONAL JOURNAL OF BIOMEDICINE

Intersections of Sociodemographic Factors and Cervical-Vaginal Infections: Implications for Preterm Birth and Abortion Outcomes

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Abstract

Background: Preterm labor and abortion, often influenced by cervical-vaginal infections, remain pressing reproductive health challenges. This study explores the interplay between socio-demographic factors, emerging pathogens like *Mycoplasma genitalium,* and their combined implications for these adverse pregnancy outcomes.

Methods and Results: The study utilized a retrospective approach based on sonographic databases, laboratory data, and clinical records of patients monitored at the Bylykbashi clinic and subsequently at the University Hospital of Obstetrics and Gynecology 'Mbreteresha Geraldine', Tirana. Data were analyzed from 2016 to 2020, involving a cohort of 1,738 pregnancies. The final group consisted of 1,482 pregnancies. These pregnancies were tracked from the time of pregnancy diagnosis until its conclusion. Vaginal secretion examinations were rigorously conducted using direct microscopy, cultures, and the A.F. GENITAL SYSTEM kit to diagnose *Ureaplasma-Mycoplasma* (UM) presence, with a focus on its association with abortions and preterm births.

Our study of 1,482 participants showed that 47.2% resided in urban areas, while 52.8% were from rural settings. Most participants fall within the 26-30 (29.6%) and 31-35 (28.1%) age groups. Regarding parity, 28.8% were primigravida, and 63% have had 12 years or less of formal education. The data showed that a significant portion (82.1%) of participants had their first sexual intercourse after the age of 18. The occurrence of urinary tract infections during pregnancy was reported by 32% of the women. UM-positive cases constituted 51.5% of the respondents. Group B *Streptococcus* and *Candida albicans* were detected in 28.5% and 47.0% of the respondents, respectively. Multivariate analyses identified younger age, early sexual debut, and primigravida status as notable risk factors for preterm births and abortions.

Conclusion: The correlation between sociodemographic factors and cervical-vaginal infections takes on significant importance in predicting maternal health outcomes, especially concerning preterm births and abortions. The heightened risk among younger individuals, those with early sexual debut, and those undergoing early onset of menarche underscores the profound influence of age and life experiences on cervical-vaginal health. Our findings related to cervical-vaginal infections emphasize the critical need for early detection, increased awareness, and prompt treatment. **(International Journal of Biomedicine. 2023;13(4):269-276.)**

Keywords: preterm birth • abortion • cervical-vaginal infections • socio-demographic factors

For citation: Bylykbashi E, Bylykbashi I, Ramosacaj E, Shpuza A, Xhetani X. Intersections of Sociodemographic Factors and Cervical-Vaginal Infections: Implications for Preterm Birth and Abortion Outcomes. International Journal of Biomedicine. 2023;13(4):269-276. doi:10.21103/Article13(4)_OA6

Abbreviations

CVIs, cervical-vaginal infections; **UM**, *Ureaplasma-Mycoplasma*; **UTIs**, urinary tract infections; **GBS**, Group B *Streptococcus*.

Introduction

Preterm labor, occurring between 20 and 37 weeks gestation, is subcategorized into early preterm (before 33 weeks) and late preterm (34 to 36 weeks), while spontaneous abortion, or abortion, is the loss of pregnancy before 20 weeks, a phenomenon that the American College of Obstetricians and Gynecologists identifies as the most common form of pregnancy loss, with estimates suggesting up to 26% of all pregnancies and 10% of clinically recognized ones ending in abortion.[1,2] Cervical-vaginal infections (CVIs), predominantly caused by *Gardnerella vaginalis*, *Candidiasis*, and *Trichomoniasis*, account for 90% of infectious-origin diseases and are prevalent issues in clinical medicine.[3] Bacterial vaginosis, while not affecting conception, is linked to a heightened risk of first-trimester abortion, and vaginal or cervical infections pose a risk, especially when the cervix is short before 28 weeks or open before 37 weeks, leading to potential preterm labor.[4,5] In addition to infections, socio-demographic factors linked to preterm birth include maternal age ≤19 years or over 35, immigrant status, education level ≤ secondary studies, residence in large cities, and single mothers or those cohabitating outside marriage, especially in countries where such cohabitation isn't common.[6,7] Additionally, higher odds of pregnancy termination are observed among adolescent girls and young women (AGYW), those cohabiting or married, regular radio and television consumers, and those in high literacy communities, while AGYW with three or more births and those with secondary/higher education have reduced termination odds.[8] Another risk is posed by *Mycoplasma genitalium* (*Mgen*), which is emerging as a cause of sexually transmitted infections, while Ureaplasma species are linked to urologic, gynecologic, and obstetric issues in men, women, and neonates.[9]

This study explores the interplay between socio-demographic factors, emerging pathogens like *Mgen*, and their combined implications for these adverse pregnancy outcomes.

Materials and Methods

The study utilized a retrospective approach based on sonographic databases, laboratory data, and clinical records of patients monitored at the Bylykbashi clinic and subsequently at the University Hospital of Obstetrics and Gynecology 'Mbreteresha Geraldine', Tirana. Data were analyzed from a 5-year period, from 2016 to 2020, involving a cohort of 1,738 pregnancies.

Exclusion criteria were a history of abortions or premature births in previous pregnancies, a subseptate or septate uterus, fibromyomas, age <18 or >40 years, infectious diseases, multiple pregnancies, conception through in IVF, hypertension, diabetes.

After these exclusions, which totaled 256 pregnancies, the final group consisted of 1,482 pregnancies. These pregnancies were tracked from the time of pregnancy diagnosis until its conclusion.

Data Collection

The sonographic report indicated that the tracked pregnancies ranged from 7 to 40 weeks of gestation. Gestational age was primarily determined based on the first date of the last menstrual cycle and adjusted accordingly with the sonographic gestational age. Detailed patient records were utilized to extract data for this study based on comprehensive medical history and a questionnaire. Complementary laboratory examinations began upon the first ultrasound confirming the embryo's presence, fetal heartbeats, and adjustments to the last menstrual cycle date by sonographic gestational age, Routine examinations included a complete blood count, biochemical tests with hepatic and renal markers, glucose levels, TORCH testing, complete urine analysis, and vaginal swabs. Any patient who would be disqualified from the study based on blood test results was identified.

Vaginal Secretion Examination

For the examination of vaginal secretions, samples were collected by physicians. The woman was placed in a lithotomy position on a gynecological bed. A sterile, unlubricated speculum was introduced to expose the cervix. Sterile swabs collected material from the posterior fornix for direct microscopy, cultures, and endocervical sampling for *Ureaplasma-Mycoplasma* (UM) testing. Concurrently, vaginal pH was assessed using litmus paper. Samples prepared with swabs immediately underwent direct microscopy, culture, and suitable UM terrains. Culture secretions were incubated for 48 hours in blood agar and McConkay mediums. For UM, the A.F. GENITAL SYSTEM kit was used. The material collected with a sterile endocervical swab was suspended in a physiological solution from the kit for 5 minutes. Afterward, the swab was gently rotated in the vial walls to obtain a homogeneous material. Using a pipette, 0.2 mL of this material was placed in each of the 24 wells of the culture medium. These contained biochemical substrates and antibiotics necessary for researching, identifying, and performing an antibiogram for each microorganism isolated from the urogenital tract. They were then covered with a drop of oil and a matching lid and incubated at 36±1°C for 18-24 hours. For cases suspected of *Mgen* or *Ureaplasma* presence, the kit underwent additional incubation for 24 hours. The wells testing for UM presence contained urea and arginine substrates. This test provided semi-quantitative data on the isolated urogenital microorganisms, particularly for the UM species. Results obtained from the A.F. GENITAL SYSTEM kits were consistent with those acquired from traditional cultures for UM identification. Antimicrobial susceptibility testing provided data on sensitivity to nine antimicrobials: Tetracycline, Doxycycline, Pefloxacin, Ofloxacin, Erythromycin, Clarithromycin, Minocycline, Vancomycin, Clindamycin.

Direct Microscopy

Direct microscopy was conducted using a Bresser Researcher ICD microscope, specifically manufactured for biological studies. It featured two light sources that can be

superimposed as needed and adjustable dioptrics depending on the examiner's eye. The Amsel criteria were used to judge the presence of *Gardnerella* or *Trichomoniasis*, even though the kit itself included these measures.

Testing Schedule

All pregnant participants were tested for UM during weeks 7-12 and then again during weeks 21-24. The kit was repeated for all the women who tested positive. Treatment was based on antimicrobial sensitivity according to readings from the A.F. genital kit, with adjustments made based on the gestational period's requirements. Clinical and sonographic data integration results regarding UM positivity or negativity were combined with sonographic and anamnestic data from accompanying clinical records.

Incidence Calculation

After recording all positive UM cases, we determined the incidence of this infection within the population. Factors such as place of residence (origin) were considered to understand the variable's impact on the development of infection and its subsequent impact on abortions and preterm births.

Clinical Record Data

From the databases and clinical records, the incidence of preterm births and abortions was determined. These figures were then compared between the population that tested positive for UM and those that tested negative. This data also provided information about women who showed symptoms and those who were asymptomatic when samples were taken. From the clinical record data, 75% of women reported symptoms like dysuria, local itching, recurrent urinary infections, and changes in vaginal secretion consistency during sample collection.

Treatment Protocol

For all patients who tested positive in the first trimester, a regimen was applied, which involved clindamycin ointment, vitamin C supplementation, and interventions to acidify vaginal pH. Treatment adjustments were made based on symptoms. Practices such as vaginal douching were discouraged, and patients were advised to abstain from sexual activities to prevent potential infection transmission through sperm. In cases diagnosed with *Gardnerella vaginalis* or *Trichomonas vaginalis* presence, vitamin C treatment was preceded by vaginal ovules containing metronidazole. After completing treatment, the A.F. Genital System kit was used again to check for microbial eradication.

Statistical Analysis Methodology

Statistical analysis was performed using statistical software package SPSS version 21.0 (SPSS Inc, Armonk, NY: IBM Corp). Data Segregation: Data from clinical records were separated into demographic and general population data. A binomial treatment of the studied variables was chosen. Data Presentation and Analysis: Discrete data were presented in absolute value and percentage. Differences for discrete data were analyzed using the Chi-square test. Casual relationships between the dependent variable (preterm birth vs. term birth) and the independent variables were analyzed using binary and multivariate logistic regression, controlling for sociodemographic and obstetric confounders. For every variable, odds ratios (OR) and their 95% confidence intervals (CI) were presented. The Chi-square test and Fisher's exact test were used to determine statistically significant differences for the studied subjects and to explicitly determine the impact of UM for each event in the study. A probability value of $P \leq 0.05$ was considered statistically significant.

Results

Table 1 highlights key attributes of participants: 47.2% reside in urban areas, while 52.8% are from rural settings. Most participants fall within the 26-30 (29.6%) and 31-35 (28.1%) age groups. In terms of parity, 28.8% are primigravida (first-time pregnant), while a significant 71.2% are non-primigravida. Regarding education, 63% have had 12 years or less of formal education, contrasting with 37% who have pursued more than 12 years. Only 8.3% reported smoking, and a minor 10.7% consumed alcohol, while the majority refrained from both habits (Table 1).

Table 1.

Sociodemographic and Behavioral Characteristics of the Study Population.

Variables	n	%
Residence		
Urban	700	47.2
Rural	782	52.8
Age groups		
18 – 20	272	18.4
21 - 25	324	21.9
26 – 30	439	29.6
31 – 35	416	28.1
36 – 40	31	2.1
Parity		
Primigravida	427	28.8
Non-primigravida	1055	71.2
Education level		
≤12 years	933	63.0
> 12 years	549	37.0
Smoking		
Yes	123	8.3
No	1359	91.7
Alcohol consumption		
Yes	158	10.7
No	1324	89.3

Table 2 provides an analysis of health practices among our population. It delves into the onset of menarche, revealing that 35% of the respondents experienced it before the age of 12, while the majority (65%) underwent menarche after this age. Additionally, the data shows that a significant portion (82.1%) of participants had their first sexual intercourse after the age of 18. When exploring the number of partners, the overwhelming majority (81%) reported having three or

fewer partners. The occurrence of UTIs during pregnancy was reported by 32% of the women. The table further uncovers that 11.9% of the women used antibiotics before conception, while 29% used hormonal therapy in the same period. Additionally, the practice of vaginal douching was limited, with only 10% engaging in the activity. Interestingly, a significant 59% of the respondents reported engaging in oral sex. Lastly, regarding hygiene practices, 78% of the participants regularly used daily pads (Table 2).

Table 2.

Health Practice Assessment

Variables	n	%	95%CI	*P*-value
Onset of Menarche				
< 12 years	519	35.0	32.6–37.5	<0.01
> 12 years	963	65.0	62.5–67.4	
First Intercourse				
≤ 18 years	266	17.9	15.9–19.9	<0.01
> 18 years	1216	82.1	80.0–84.0	
Number of Partners				
≤ 3 partners	1201	81.0	71.9–82.9	<0.01
> 3 partners	281	19.0	17.0–21.0	
UTI during Pregnancy				
Yes	474	32.0	29.6–34.4	<0.01
No	1008	68.0	65.6–70.0	
Antibiotic Use Preconception				
Yes	177	11.9	10.3–13.6	<0.01
No	1305	88.1	86.3–89.7	
Hormonal Therapy Preconception				
Yes	430	29.0	26.7–31.4	<0.01
No	1052	71.0	68.6–73.2	
Vaginal Douching				
Yes	148	10.0	8.5–11.6	<0.01
No	1334	90.0	88.3–91.4	
Engaging in Oral Sex				
Yes	874	59.0	56.5– 61.4	<0.01
No	608	41.0	38.5–43.5	
Regular Use of Daily Pads				
Yes	1156	78.0	75.8–80.0	<0.01
No	326	22.0	19.9–24.1	

Table 3 provides a comprehensive breakdown of the prevalence of various CVIs among the studied population. UM-positive cases constituted 51.5% of the respondents, while 48.5% tested negative, with a statistically significant difference (P<0.01). Group B *Streptococcus* (GBS) was detected in 28.5% of the participants, leaving the majority, 71.5%, without this bacterial presence, showing a statistically significant variation. *Chlamydia* was notably less prevalent, with only 2.1% testing positive, as opposed to a whopping 97.9% testing negative, and this difference was statistically significant (P<0.01). Lastly, *Candida albicans*, a common fungus causing yeast infections, was present in 47.0% of the respondents, with the remaining 53.0% being unaffected, and this distinction was also statistically significant (P<0.01).

Tabel 3.

Cervical-vaginal infections assessment.

Variables	n	%	95%CI	*P*-value
Presence of UM+				
Yes	763	51.5	48.9–54.0	<0.01
No	719	48.5	45.9–51.0	
Presence of GBS				
Yes	422	28.5	26.2–30.8	<0.01
No	1060	71.5	69.1–73.8	
Presence of *Chlamydia*				
Yes	31	2.1	1.4–2.9	<0.01
No	1451	97.9	97.0–98.6	
Presence of *Candida albicans*				
Yes	696	47.0	44.4–49.6	<0.01
No	786	53.0	50.4–55.6	

In the univariate analysis of factors potentially associated with preterm birth (UM+), several variables were identified. Women aged ≤20 years demonstrated a 1.9-fold increased risk of preterm birth (95% CI: 1.4–2.6, P<0.01). Those with an education level of ≤12 years faced a 2.2-fold elevated risk (95% CI: 1.8–2.8, P<0.01). Primigravida women showed a slight elevation in risk, although this wasn't statistically significant (OR=1.2, 95% CI: 0.9–1.5, P=0.1). Smoking during pregnancy increased the risk by 1.5 times, but the result was borderline significant (95% CI: 0.9–2.3, P=0.09). Early initiation of intercourse (≤18 years) presented a 1.5-fold increased risk (95% CI: 1.1–1.9, P=0.01). Intriguingly, women with more than three partners had a significantly decreased risk (OR=0.1, 95% CI: 0.05–1.3, P<0.01). A prominent risk was observed with the presence of *Candida albicans*, showing an 8.7-fold increase (95% CI: 6.8–11, P<0.01). Early onset of menarche (<12 years), vaginal douching, engaging in oral sex, and regular use of daily pads were all found to significantly elevate the risk of preterm birth. Several other factors like UTIs during pregnancy, *Chlamydia* presence, and preconception hormonal therapy showed elevated risks, though not reaching statistical significance (Table 4).

In a multivariate logistic regression analysis, a significant association was observed between UM+ and several factors.

Notably, individuals younger than 20 had an OR of 1.9 (95% CI: 1.4–2.6, *P*<0.01). Additionally, those who experienced their first sexual intercourse at the age of 18 presented an OR of 1.4 (95% CI: 1.1–1.9, *P*<0.01). Furthermore, the onset of menarche before the age of 12 was linked to an OR of 1.3 (95% CI: 1.0–1.6, *P*<0.01) (Figure 1).

Table 4.

Univariate Logistic Regression Analysis of Risk Factors Associated with Preterm Birth (UM+)

Variables	UM+ (n)	%	OR	95%CI	*P*- value
Age					
≤ 20 years (n=272)	59	21.7	1.9	1.4–2.6	<0.01
Education level					
≤ 12 years (n=933)	217	23.3	2.2	1.8–2.8	<0.01
Parity					
Primigravida (n=427)	144	33.7	1.2	0.9–1.5	0.1
Smoking					
Yes (n=123)	28	22.8	1.5	0.9–2.3	0.09
First intercourse					
≤18 years (n=266)	87	32.7	1.5	1.1–1.9	0.01
Nr. of partners					
> 3 partners (n=281)	22	7.8	0.1	0.05–1.3	<0.01
UTI during Pregnancy					
Yes (n=474)	104	21.9	1.2	0.9–1.6	0.1
Antibiotic Use Preconception					
Yes (n=177)	69	39.0	0.9	0.7–1.3	0.6
Presence of *Chlamydia*					
Yes (n=31)	8	25.8	2	0.9– 4.5	0.09
Presence of GBS					
Yes (n=422)	107	25.4	1.3	0.9–1.6	0.07
Presence of *Candida albicans*					
Yes (n=696)	542	77.9	8.7	6.8–11	<0.01
Onset of Menarche					
< 12 years (n=519)	236	45.5	1.3	1.0–1.6	0.03
Hormonal Therapy Preconception					
Yes (n=430)	85	19.8	0.2	0.15–0.26	<0.01
Vaginal Douching					
Yes (n=148)	111	75.0	2.6	1.8–3.9	<0.01
Engaging in Oral Sex					
Yes (n=874)	533	61.0	1.9	1.5–2.3	<0.01
Regular Use of Daily Pads					
Yes (n=1156)	820	70.9	2.5	1.9–3.2	<0.01

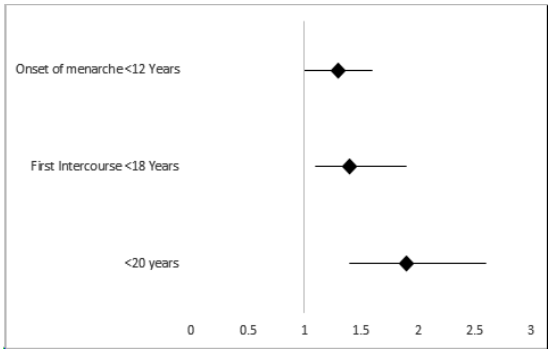

Fig. 1. *Associations between UM+ and Age, Age at First Intercourse, and Age at Menarche in a Multivariate Logistic Regression Analysis*

In the univariate logistic regression analysis presented in Table 5, examining specific population characteristics and their association with abortion, several findings were observed. Women aged ≤20 years had a 2.2-fold increased risk of abortion, albeit not statistically significant (95% CI: 0.40–12.25, *P*=0.3). Those with an education level of ≤12 years presented a 1.2-fold increased risk (95% CI: 0.41–6.45, *P*=0.8). Primigravida women demonstrated a notable 5.0-fold heightened risk, nearing significance (95% CI: 0.90–27.28, *P*=0.06). Smoking was associated with a 2.2-fold rise in abortion risk (95% CI: 0.25–19.15, *P*=0.4). Women whose first sexual encounter occurred at ≤18 years displayed a 2.3-fold increased risk (95% CI: 0.41–15.59, *P*=0.3). A distinct finding was the significant association between abortion and having more than three partners, showing an 8.7-fold amplified risk (95% CI: 1.57–47.5, *P*=0.01). Women who experienced menarche before 12 years of age had a reduced risk (OR=0.4, 95% CI: 0.04–3.17, *P*=0.3). Other factors such as UTIs during pregnancy, antibiotic use before conception, hormonal therapy before conception, presence of GBS, *Chlamydia*, *Candida albicans*, vaginal douching habits, oral sex activities, and regular use of daily pads were also assessed, but they did not exhibit statistically significant ties with abortion in this analysis.

In the results of the multivariate logistic regression analysis, three significant independent risk factors for abortion were identified. First, individuals with more than three partners had an OR of 8.7 (95% CI: 1.57–47.5, *P*<0.01). Secondly, primigravida women had an increased risk, with an OR of 6.8 (95% CI: 5.6–13.2, *P*<0.01). Lastly, those who had their first intercourse before the age of 18 presented an OR of 3.3 (95% CI: 1.4–16.7, *P*<0.01) (Figure 2).

Discussion

The complex interplay of sociodemographic factors and CVIs plays a pivotal role in maternal health outcomes, particularly concerning preterm births and abortions. This article seeks to explore these intersections and delve into their implications, providing a nuanced understanding of the underlying causes and potential preventive measures.

Table 5.

Specific Population Characteristics and Abortions. Univariate Logistic Regression Analysis.

Variables	Abortion n (%)	OR	95%CI	*P*-value
Age				
≤ 20 years (n=272)	2 (0.74)	2.2	0.40–12.25	0.3
Education level				
≤ 12 years (n=933)	4 (0.43)	1.2	0.41–6.45	0.8
Parity				
Primigravida (n=427)	4 (0.94)	5.0	0.90–27.28	0.06
Smoking				
Yes (n=123)	1 (0.81)	2.2	0.25–19.15	0.4
First intercourse				
≤18 years (n=266)	2 (0.75)	2.3	0.41–15.59	0.3
Nr. of partners				
> 3 partners (n=281)	4 (1.42)	8.7	1.57–47.5	0.01
Onset of Menarche				
< 12 years (n=519)	1 (0.19)	0.4	0.04–3.17	0.3
UTI during Pregnancy				
Yes (n=474)	2 (0.42)	1.1	0.19–5.82	0.9
Antibiotic Use Preconception				
Yes (n=177)	1 (0.56)	1.5	0.17–2.71	0.7
Hormonal Therapy Preconception				
Yes (n=430)	2 (0.47)	1.2	0.22–6.7	0.8
Presence of GBS				
Yes (n=422)	4 (0.95)	5.1	0.92–27.74	0.06
Presence of *Chlamydia*				
Yes (n=31)	1 (3.23)	9.6	1.09–85.04	0.04
Presence of *Candida albicans*				
Yes (n=696)	4 (0.57)	2.3	0.41–12.45	0.3
Vaginal Douching				
Yes (n=148)	1 (0.68)	1.8	0.20–15.58	0.5
Engaging in Oral Sex				
Yes (n=874)	2 (0.23)	0.3	0.06–1.89	0.2
Regular Use of Daily Pads				
Yes (n=1156)	3 (0.26)	0.3	0.05–1.39	0.1

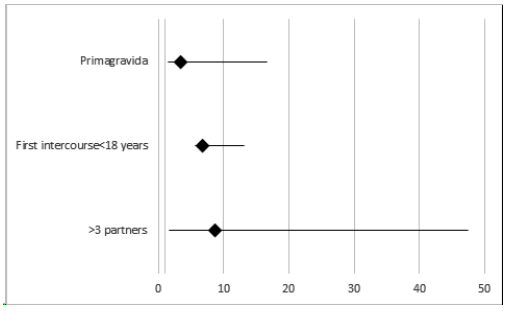

Fig. 2. *Independent Risk Factors for Abortion: ORs and 95% CIs from Multivariate Logistic Regression*

The age concentration of patients in the 26-35 bracket seems to correspond with global trends. According to WHO evidence, women in many regions are increasingly delaying childbearing to their late twenties and early thirties.[10] The marked difference in parity, with a substantial proportion being non-primigravida, might suggest cultural or health system factors influencing multiple pregnancies. The educational breakdown aligns with many global settings where completion of secondary education remains the most common highest attainment. The relatively low rates of smoking and alcohol consumption are encouraging and might reflect sociocultural norms regarding these behaviors, especially among potential mothers.[11]

A notable observation is the age at onset of menarche, with a significant 35% of respondents experiencing it before the age of 12, like other studies that indicate almost the same age at menarche's onset.[12] The fact that most participants initiated sexual activity after 18 might be reflective of cultural norms, educational efforts, or both, and this observation aligns with other studies emphasizing the trend toward delayed first sexual experiences in some demographics.[13] The reported number of partners and the relatively low occurrence of UTIs provide crucial insights into sexual health and risks. The data regarding antibiotic and hormonal therapy use preconception is noteworthy, as these factors may have implications for reproductive health.[14] The limited practice of vaginal douching is also linked with sociocultural norms, even though it resonates with global health advice cautioning against frequent douching.[15] The high percentage of respondents partaking in oral sex provides a dimension to understanding broader sexual health behaviors and practices. Lastly, the prevalent use of daily pads underscores the importance of understanding hygiene practices and their potential health implications.[16]Top of Form

Notably, more than half of the participants exhibited UM-positive cases, a prevalence rate that may be compared to other regional statistics for context.[17] The presence of GBS in over a quarter of participants warrants attention, especially given the potential implications for neonatal health. Conversely, the remarkably low prevalence of *Chlamydia*, though encouraging, suggests a possible success of prior awareness or intervention campaigns, yet it is essential to evaluate this in relation to other populations. As for *Candida albicans*, its presence in nearly half of the participants aligns with its known ubiquity and its common occurrence in many women; this prevalence rate agrees with global averages and emphasizes the need for continuous monitoring and awareness.[18] The marked differences in the prevalence rates of each infection underscore the diverse nature of CVIs and their varied presence in this specific population.

Considering the broader literature, our results elucidate key associations between UM+ and certain demographic and sexual health factors. For instance, individuals under 20 demonstrated a heightened risk, which aligns with previous studies indicating increased susceptibility in younger populations.[19] Likewise, the onset of sexual activity by the age of 18 has been previously linked to various health outcomes in the literature. Interestingly, our study further illuminates the

correlation between early onset of menarche (before age 12) and UM+, a connection that has been touched upon in prior research but necessitates more in-depth exploration.[20]

In comparison with existing literature, our findings underscore several significant independent risk factors for abortion. The observed increased risk in individuals with multiple sexual partners aligns with previous studies that highlight the correlation between the number of partners and adverse reproductive outcomes.[21] Additionally, the elevated risk for individuals who initiated sexual activity before the age of 18 complements past research, emphasizing the potential reproductive health consequences of early sexual debut.[22] Further studies are necessary to elucidate the potential mechanisms underlying these associations.

Although this study revealed some valuable information, it has several limitations. The study, being retrospective, relied on previously collected data, potentially making it susceptible to the accuracy and quality of past record-keeping. Its numerous exclusion criteria, such as the omission of those under 18 or over 40 and those who had IVF, could limit the generalizability of the results to a broader population. Furthermore, the research was centered in Tirana, Albania, which may not represent outcomes in other regions. The heavy reliance on self-reported data, like the date of the last menstrual cycle, introduces the possibility of recall bias. The use of a single kit for U-M testing and the Bresser Researcher ICD microscope for direct microscopy might have inherent biases or limitations in their respective methodologies.

Drawing from our extensive exploration, the intersections of sociodemographic factors and CVI emerge as critical determinants in the landscape of maternal health, particularly in the context of preterm births and abortions. As we conclude, it becomes paramount to emphasize the potential of this intersectional understanding in tailoring health interventions and policies. The nuances unraveled in this study underline the importance of a holistic approach, one that considers both sociodemographic intricacies and biological indicators, to mitigate risks and enhance maternal outcomes in diverse populations. Future endeavors in this realm hold the promise of not only enriching our comprehension but also fortifying preventive and therapeutic strategies in maternal healthcare.

Conclusion

Considering the comprehensive insights derived from our study, the correlation between sociodemographic factors and CVIs takes on significant importance in predicting maternal health outcomes, especially concerning preterm births and abortions. The heightened risk among younger individuals, those with early sexual debut, and those undergoing early onset of menarche underscores the profound influence of age and life experiences on cervical-vaginal health. Our findings related to CVIs—such as the elevated incidence of UM-positive cases, the presence of CBS, and the prevalence of *Candida albicans*—emphasize the critical need for early detection, increased awareness, and prompt treatment. These outcomes not only highlight potential causes for preterm births and abortions but also illustrate the deep interplay between individual behaviors, sociodemographic backgrounds, and biological factors in determining reproductive health outcomes. As we move forward in designing more tailored interventions, integrating these crucial findings will be vital in shaping strategies that not only target CVIs but also directly address the associated risks of preterm births abortions.

Competing Interests

The authors declare that they have no competing interests.

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International Journal of Biomedicine 13(4) (2023) 277-280
http://dx.doi.org/10.21103/Article13(4)_OA7

ORIGINAL ARTICLE

Ophthalmology

INTERNATIONAL JOURNAL OF BIOMEDICINE

A Comparative Study on Surgically Induced Astigmatism after Phacoemulsification and Its Correlation with the Central Corneal Thickness

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Abstract

***Background*:** Cataracts being a disease with a very high prevalence, have been treated with various surgical and conservative forms for centuries. The aim of our study was to determine the correlation between central corneal thickness (CCT) and post-operative astigmatism after phacoemulsification.

***Methods and Results*:** This prospective, observational study was carried out in the Clinic of Ophthalmology at the University Clinical Center of Kosovo. The study included 101 eyes of patients who underwent cataract surgery with phacoemulsification for treatment and intra-ocular lens (IOL) implantation. Patients were divided into two groups, depending on the central thickness of the cornea. Group 1 included 29 patients who had CCT <550 µm and Group 2 included 72 patients who had CCT ≥550 µm. The mean age of patients in Group 1 and Group 2 was 69.0±10.5 years and 70.1±10.8 years, respectively, without significant difference between groups. The right eye was affected in 54.6% of cases. The patients were followed up for the evaluation of post-operative astigmatism on Day 7, two weeks, and two months after the surgery. Astigmatism was lower in Group 2 than in Group 1 at all stages of the examination but without a statistically significant difference. We did not find significant correlations between the CCT and astigmatism after phacoemulsification.

Conclusion: Results of our study show no significant correlation between CCT and SIA after cataract surgery with phacoemulsification. **(International Journal of Biomedicine. 2023;13(4):277-280.)**

Keywords: central corneal thickness • phacoemulsification • surgically induced astigmatism

For citation: Shoshi F, Spahiu K, Shoshi F. A Comparative Study on Surgically Induced Astigmatism after Phacoemulsification and Its Correlation with the Central Corneal Thickness. International Journal of Biomedicine. 2023;13(4):277-280. doi:10.21103/Article13(4)_OA7

Abbreviations

CCT, central corneal thickness; **DCyl**, diopter cylinder; **ECCE**, extracapsular cataract extraction; **IOL**, intra-ocular lens; **OCT**, optical coherence tomography; **SIA**, surgically induced astigmatism.

Introduction

Cataracts being a disease with a very high prevalence, have been treated with various surgical and conservative forms for centuries.(1) Cataracts are the leading cause of vision loss in middle- and low-income countries, thus causing 50% of blindness cases in these countries and 5% in developed countries.(2) Every patient who will undergo cataract surgery must undergo a detailed ophthalmological examination, which includes measurement of the visual acuity, examination with the slit-lamp, tonometry, ultrasound, and pachymetry. Ophthalmological disorders at the time of examination can impact visual acuity (vision). Although most cataract surgeries go without any complications, any potential problem plays a role if the surgery is complicated or prolonged.(1) Preoperative determination of corneal topography is a useful investigation in cataract surgery planning. Corneal topography can be used as an alternative to keratometry because it provides a

representative measurement of the corneal curvature needed to calculate the IOL. Second, corneal topography is important if the degree and location of pre-existing astigmatism are known so that they can be considered during surgery.[1,3] Determination of corneal topography facilitates meeting the expectations of surgery within 0.5 diopters of emmetropia with minimal astigmatism.[4,5] In planning the correction of astigmatism, it is not only important to measure the corneal component of astigmatism but also to reduce surgically induced astigmatism (SIA). Extracapsular cataract extraction (ECCE) causes a larger amount of astigmatism than phacoemulsification.[6] Although manual small-incision ECCE has been proposed to reduce costs and allow a faster recovery than traditional ECCE, phacoemulsification still causes less SIA. It is superior to manual small-incision cataract surgery in uncorrected visual acuity. It has been suggested that the visual rehabilitation, corneal endothelial cell loss, and complication rates after manual small-incision cataract surgery are comparable to those of phacoemulsification, but more SIA is induced, resulting in less desirable uncorrected visual acuity.[7]

Cataract surgical treatment with phacoemulsification proves to be superior to classical ECCE. The literature review of 11 clinical trials comparing these two methods of cataract treatment shows that surgical treatment of cataracts with phacoemulsification is superior to extracapsular treatment both in visual acuity of patients and in fewer intra-operative and post-operative complications.[4] Considering the size of the incision, the degree of astigmatism in cases treated with phacoemulsification is lower than in cases treated with the classic ECCE method; visual acuity was significantly better in patients treated with phacoemulsification and the degree of astigmatism was lower at all measurement intervals than in the ECCE-treated group.[5] Since the correlation between CCT and the degree of post-operative astigmatism after phacoemulsification is still a new aspect in the field of cataract and refractive surgery, especially in our country, the main aim of our study was to determine the correlation between CCT and post-operative astigmatism after phacoemulsification.

Materials and Methods

This prospective, observational study was carried out in the Clinic of Ophthalmology at the University Clinical Center of Kosovo. The study included 101 eyes of patients who underwent cataract surgery with phacoemulsification for treatment and IOL implantation. This procedure is routine in the Department of Ophthalmology for treating cataracts and is applied to more than 99% of patients. Therefore, the research is only observational.

The data was collected within a period of 12 months from July 2022 to June 2023, and the cases were treated by surgeons with more than 10 years of experience. This procedure has started to be applied in UCCK since 2015.

Exclusion criteria

- Patients who have had earlier interventions in the eye in which the surgery with phacoemulsification would be performed, such as phototherapeutic keratectomy, photorefractive keratectomy, or laser-assisted in situ keratomileusis

- Patients with diseases/disorders of the cornea, such as degenerative diseases, previous injuries in the eye where the intervention would be performed, keratoconus, or diabetes

The study included 101 patients (54[53.5%] men and 47[46.5%] women) with cataracts. Before the surgery (preoperative) upon admission to the Clinic of Ophthalmology, the patients underwent a detailed ophthalmological examination, which included the determination of the visual acuity (vision); slit-lamp examination, B-scan ultrasound and pachymetry (measurement of CCT) as the key method of this research. After performing the pachymetry, the intra-ocular pressure was measured with a tonometer, and the data were placed on separate forms for each patient. The central thickness of the cornea was measured with OCT. Pachymetry data are saved in the computer from which the data on the corneal thickness of each patient are obtained. The astigmatism was assessed by using the auto kerato-refractometer. The measurement of the CCT was performed before the measurement of the intra-ocular pressure in order to avoid any possible trauma to the cornea that could be caused during the tonometry, which could interfere with the results of the CCT.

Patients were divided into two groups, depending on the central thickness of the cornea. Group 1 included 29 patients who had CCT <550 μm and Group 2 included 72 patients who had CCT ≥550 μm. The patients were followed up for the evaluation of post-operative astigmatism on Day 7, two weeks, and two months after the surgery.

Statistical analysis was performed using the statistical software package SPSS version 22.0 (SPSS Inc, Armonk, NY: IBM Corp). The normality of distribution of continuous variables was tested by the Shapiro-Wilk test. For the descriptive analysis, results are presented as mean (M) ± standard deviation (SD) or as median and interquartile range (IQR). For data with normal distribution, inter-group comparisons were performed using Student's t-test. The Mann-Whitney U Test was used to compare the differences between the two independent groups (for nonparametric data). Group comparisons with respect to categorical variables were performed using chi-square test. Spearman's rank correlation coefficient (r_s) was calculated to measure the strength and direction of the relationship between two variables. A probability value of $P<0.05$ was considered statistically significant.

Results

The mean age of patients in Group 1 and Group 2 was 69.0±10.5 years and 70.1±10.8 years, respectively, without significant difference between groups. The right eye was affected in 54.6% of cases (Table 1).

Table 1.

Distribution of respondents by socio-demographic characteristics.

Parameter	Group 1 (n=29)	Group 1 (n=72)	Total (n=101)	*P*-value
Gender, n (%) Male Female	13 (44.8) 16 (55.2)	41 (56.9) 31 (43.1)	54 (53.5) 47 (46.5)	0.269
Age, yrs Mean ± SD Range	69.0 ± 10.5 44 - 86	70.1 ± 10.8 38 - 92	69.8 ± 10.7 38 - 92	0.588
Eye, n (%) OD OS	14 (48.3) 15 (51.7)	43 (59.7) 29 (40.3)	57 (56.4) 44 (43.6)	0.294

Mean values of preoperative astigmatism and astigmatism on Day 7, two weeks, and two months after surgery are presented in Table 2. Astigmatism was lower in Group 2 than in Group 1 at all stages of the examination but without a statistically significant difference. We did not find significant correlations between the CCT and astigmatism after phacoemulsification (Table 3).

Table 2.

Preoperative astigmatism and astigmatism on Day 7, two weeks, and two months after surgery.

Astigmatism, dcyl	Group 1 (n=29)	Group 1 (n=72)	*P*-value
Preoperative			
Mean ± SD Range	-0.66 ± 1.16 -2.75 to 1.25	-0.15 ± 1.09 -2.75 to 1.75	0.092
Day 7 after surgery			
Mean ± SD Range	-0.74 ± 1.66 -4.0 to 2.25	-0.46 ± 1.44 -3.25 to 2.50	0.376
Two weeks after surgery			
Mean ± SD Range	-0.62 ± 1.39 -3.0 to 1.75	-0.37 ± 1.34 -3.0 to 2.50	0.395
Two months after surgery			
Mean ± SD Range	-0.59 ± 1.11 -2.25 to 1.5	-0.32 ± 1.14 -2.25 to 2.50	0.315

Table 3.

Correlations between preoperative astigmatism, SIA (on Day 7, two weeks, and two months after surgery) and CCT.

Astigmatism and CCT	Spearman Corelation		
	r_s	95% CI	*P*-value
Baseline	0.184	-0.069 to 0.416	0.141
Day 7 after surgery	0.096	-0.109 to 0.295	0.342
Two weeks after surgery	0.031	-0.176 to 0.236	0.761
Two months after surgery	0.165	-0.051 to 0.366	0.121

Discussion

SIA was one of the factors that influenced the desirable refractive outcome.[8] SIA was related to the characteristics of the incision (length, type, location, structure, etc.); however, the most significant factor was incision width.[9] As a result, cataract surgeries through small corneal incisions are increasing in popularity,[10,11] although the proper size for a truly astigmatic cataract incision has not been established. Masket et al.[12] have demonstrated that SIA with 2.2mm micro-coaxial incisions induce less astigmatism postoperatively than do traditional 3.0mm clear corneal incisions. The reduction of the incision size causes less post-operative astigmatism after cataract surgery.[13,14]

The correlation between CCT and the degree of post-operative astigmatism after phacoemulsification is still a new aspect in the field of cataract and refractive surgery, especially in our country. The results of this study could be a milestone in the development of IOL calculation and surgical incision planning based on central corneal thickness to reduce the rate of post-operative astigmatism. This approach would significantly improve post-operative outcomes and increase the quality of vision in patients. There is no relative study on the correlation between corneal thickness and SIA after vitrectomy.[15] But Woo and Lee [16] studied the effect of CCT on SIA in cataract surgery using a temporal precise corneal incision and observed that CCT could negatively influence the amount of SIA immediately postoperatively, but the correlation was not present 2 months after surgery. Results of our study show no significant correlation between CCT and SIA after cataract surgery with phacoemulsification.

Competing Interests

The authors declare that they have no competing interests.

Ethical considerations

Ethical Approval for this research was obtained from the Ethical Committee of the Kosovo Chamber of Physicians (№ 51/2021 dated 06/10/2021) and the Ethical Committee of the Faculty of Medicine, University of Prishtina (№ 13482 dated 24/12/2021).

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International Journal of Biomedicine 13(4) (2023) 281-285
http://dx.doi.org/10.21103/Article13(4)_OA8

ORIGINAL ARTICLE

Infectious Diseases

INTERNATIONAL JOURNAL OF BIOMEDICINE

Coexistence of HBsAg/Anti-HBs and HBeAg/Anti-HBe in Sudanese Patients with Chronic Hepatitis B Virus Infection: A Cross-Sectional Study

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Abstract

Background: Seroconversion of hepatitis B surface antigen (HBsAg) to hepatitis B surface antibody (anti-HBs) is a recognized goal of HBV therapy. This dynamic transition responsible for the coexistence of HBsAg and anti-HBs is rarely detected in clinical cases. However, with vaccination and the use of various antiviral drugs, as well as the development of new medical technologies, recognizing the coexistence of HBsAg and anti-HBs has become more common. In addition, mutations in viral genomes, immune status, and human genetic factors may also contribute to such coexistence. The current study was designed to determine the prevalence of the coexistence of HBsAg and anti-HBs and HBeAg and anti-HBe in CHB patients in Sudan.

Methods and Results: This was a descriptive cross-sectional study conducted in Khartoum state from November 2018 to January 2019. The study included 70 HBV-infected patients who were positive for HBsAg for more than six months. Blood samples were tested for HBsAg/Anti-HBs and HBeAg/Anti-HBe using Commercial ELISA Kits (Foresight, United Kingdom) and (PRECHEK, USA). Demographic data were collected using a structured questionnaire, and any antiviral agent and laboratory results were also recorded for each participant. The current study showed that one case (1.4%) was reactive for the coexistence of HBsAg/HBsAb and two cases (2.8%) for the coexistence of HBeAg/HBeAb. There was no statistical difference between the coexistence of HBsAg/HBsAb and HBeAg/HBeAb with age, gender, residence, and treatment status.

Conclusion: Our study indicates that the frequencies of the coexistence of HBsAg/HBsAb and HBeAg/HBeAb among Sudanese patients with chronic HBV infection were low compared to previous studies in a different population.**(International Journal of Biomedicine. 2023;13(4):281-285.)**

Keywords: hepatitis B virus • HBsAg • HBeAg • anti-HBs • anti-HBe

For citation: Hashim T, Osman N, Abd-Alazeem A, Abbas AM, Shalabi MG, Elsiddig SA, Alameen AAM, Eltayeb LB, Babker AM, Mohamed H, Suliman RS, Yassin ME. Coexistence of HBsAg/Anti-HBs and HBeAg/Anti-HBe in Sudanese Patients with Chronic Hepatitis B Virus Infection: A Cross-Sectional Study. International Journal of Biomedicine. 2023;13(4):281-285. doi:10.21103/Article13(4)_OA8

Abbreviations

anti-HBs, hepatitis B surface antibody; **anti-HBe**, HBe antibody; **anti-HBc**, total antibody to hepatitis B core antigen; **CHB**, chronic hepatitis B; **HBsAg**, hepatitis B surface antigen; **HBV**, hepatitis B virus; **HBeAg**, hepatitis B e antigen.

Introduction

Hepatitis B virus (HBV) infection is a universal public health problem and a common cause of liver cirrhosis and hepatocellular carcinoma. Globally, a probable 257 million people are living with chronic HBV, which affects primarily many African countries and the western Pacific region, according to the WHO.[1]

HBV clearance is classically characterized by the occurrence of hepatitis B surface antibody (anti-HBs) homologous to hepatitis B surface antigen (HBsAg), which contains several important antigenic epitopes, in particular a determinant that spans amino acids 124-147 within its major hydrophilic region (MHR).[2-4] Thus, anti-HBs and HBsAg are characteristically not recognized in sera of people with present HBV infection in routine clinical practice.[5]

However, several studies have depicted the manifestation of HBsAg linked with anti-HBs in CHB patients.[6-10] With variable rates of coexisting anti-HBs and HBsAg in chronic patients, the HBsAg carriers were projected to be 2.43%-8.9%.[5,11-14] Further, the mechanism underlying the advent of anti-HBs in patients with chronic hepatitis B dregs is indistinct. Still, numerous studies have disclosed that HBV mutations could be attributed to the selection of immune escape mutations.[10,12,14-17]

The key methods widely used for testing hepatitis B serology are the tests for detecting HBsAg and HBeAg and their matching with antibodies anti-HBe, anti-HBc IgM, anti-HBs, and anti-HBc (total). A previous study showed that succeeding infection with HBV naturally, HBsAg can be noticeable in serum during the cultivation period of 3-5 weeks before the presence of clinical symptoms and continues for 2-4 weeks after the augmentation of transaminase level; it is not detected in 2-6 months as the patient recovers and after a window period, protective anti-HBs antibody appears. The perseverance of HBsAg beyond six months after acute infection is putative evidence of chronic infection. Occasionally, the seroconversion of HBeAg to anti-HBe consensuses with the reduction or normalization of serum ALT concentration and a very low level of HBV duplication. However, recent studies have established that the serum HBV DNA concentration is not associated with the presence or absence of HBeAg.[18]

Meanwhile, the marker for the resolution of acute infection can be identified by the appearance of the antibodies to HBeAg, which is used to monitor treatment, and the appearance of anti-HBe differentiates between the two stages. A negative result of the HBeAg may designate an early severe infection before the peak of viral duplication or early recovery when HBeAg has dropped below detectable levels. It is important to state a subset of CHB patients is not obvious HBeAg in serum but is positive for anti-HBe; these types of patients may also be positive for serum hepatitis B virus DNA.

Additionally, when treating CHB patients, the antigen/antibody seroconversion can be used as a marker of virological response.[19] The coexistence of HBsAg and anti-HBs contains an atypical serological profile in patients with HBV infection. Hence, in the presence of a circulating immune complex with optimal proportion and new subtype infection, the coexistence of anti-HBs and HBsAg might reveal the strictness of liver disease and the active replication or reactivation of the virus.[20] It is unusual for patients with prolonged HBV to relate to more advanced liver diseases.[12]

According to many publications, Sudan is classified among the countries with high HBV seroprevalence endemicity, with infection rates ranging from 6.8% in central to 26% in southern Sudan, and this infection is common among patients hospitalized with hepatocellular carcinoma. Also, there are many challenges facing healthcare workers in the country in combating severe health problems like hepatitis infection, cancer, HIV, and Dengue virus.[21-25]

The current study was designed to determine the prevalence of the coexistence of HBsAg and anti-HBs and HBeAg and anti-HBe in CHB patients in Sudan.

Materials and Methods

This was a descriptive cross-sectional study conducted in Khartoum state from November 2018 to January 2019. The study included 70 HBV-infected patients (54[77.1%] male and 16[22.9%] female; age range from 19 to 65 years with mean age of 34.35 years) who were positive for HBsAg for more than six months, attending Saba Medical Complex, Ibrahim Malik Teaching Hospital, and The Hospital of ibn Sina in Khartoum. The blood samples (2.5 ml in a plain container) were collected, let to clot, and then separated to obtain serum. All samples were tested for HBsAg/Anti-HBs and HBeAg/Anti-HBe using Commercial ELISA Kits (Foresight, United Kingdom) and (PRECHEK, USA). Demographic data were collected using a structured questionnaire, and any antiviral agent and laboratory results were also recorded for each participant.

Statistical analysis was performed using the statistical software package SPSS version 20.0 (SPSS Inc, Armonk, NY: IBM Corp). Baseline characteristics were summarized as frequencies and percentages. Group comparisons were performed using chi-square test or, alternatively, Fisher's exact test when expected cell counts were less than 5. A probability value of $P<0.05$ was considered statistically significant.

The study was approved by the Ethics Committee at the Al Neelain University.

Results

Of 70 patients with chronic HBV infection, there were 37(52.8%) under specific HBV treatment, while 33(47.1%) were treated naïvely. The participants were classified into two groups, from 19 to 40 years and from 41 to 65 years, which were distributed as 58/70(82.9%) and 12/70(17.1%),

respectively. Out of the 70 participants, 61(87.1%) resided in Khartoum, 2(2.8%) in Omdurman, and 7(10%) in North Khartoum (Bahri).

The current study showed that one case (1.4%) was reactive for the coexistence of HBsAg/HBsAb and two cases (2.8%) for the coexistence of HBeAg/HBeAb. Among 70 patients, 6(8.6%) carried HBeAg and 58(82.9%) carried HBeAb. There was no statistical difference between the coexistence of HBsAg/HBsAb and HBeAg/HBeAb with age, gender, residence, and treatment status (Tables 1 and 2).

Table 1.

Coexistence of HBsAg/HBsAb regarding age, gender, residence, and treatment status.

Variable		HBsAg/HBsAb		*P*-value
		Reactive n (%)	Non-reactive n (%)	
Age	19—40	0 (0)	58 (82.9)	0.171
	41—65	1 (1.4)	11 (15.7)	
Gender	Male	1 (1.4)	53 (75.7)	1.0
	Female	0 (0)	16 (22.8)	
Treatment	Specific HBV treatment	0 (0)	37 (52.8)	0.471
	Treatment-naïve	1 (1.4)	32 (45.7)	
Residence	Khartoum	1 (1.4)	60 (85.7)	0.928
	Bahri	0 (0)	7 (10.0)	
	Omdurman	0 (0)	2 (2.8)	

Table 2.

Coexistence of HBeAg/HBeAb regarding age, gender, residence, and treatment status.

Variable		HBeAg/HBeAb		*P*-value
		Reactive n (%)	Non-reactive n (%)	
Age	19—40	2 (2.8)	56 (80.0)	1.0
	41—65	0 (0)	12 (17.1)	
Gender	Male	2 (2.8)	52 (74.3)	1.0
	Female	0 (0)	16 (22.8)	
Treatment	Specific HBV treatment	1 (1.4)	36 (51.4)	1.0
	Treatment-naïve	1 (1.4)	32 (45.7)	
Residence	Khartoum	2 (2.8)	59 (84.3)	0.859
	Bahri	0 (0)	7 (10.0)	
	Omdurman	0 (0)	2 (2.8)	

Discussion

The coexistence of anti-HBs and HBsAg in patients with HBV infection is uncommon[26] but may be associated with more progressive liver diseases.[12] The HBeAg is a significant marker of viral replication in chronic infection, infectivity, and constant liver injury.

The antibody to HBeAg is noticeable as HBeAg vanishes from the serum, and the existence of anti-HBe is linked with the probability of impulsive resolution of severe infection.

In patients enduring HBV infection, the downfall of HBeAg and the gaining anti-HBe tend to be allied with biochemical and histological upgrading.[27] Previous studies have shown the prevalence of coexistence of anti-HBs with HBsAg and anti-HBe with HBeAg in patients with chronic hepatitis B infection, with significant variation in many different countries. Our study found that the frequency of the coexistence of HBsAg and HBsAb was 1.4%, which is comparable to a previous study on patients with chronic HBV infection by Wang et al.,[28] who found that the dominance of atypical serological pattern was 2.93% (122/4169). The prevalence progressively increased with age from 40 to 70 years old. In addition, the rate of HBeAg positive and detectable HBV DNA were both significantly higher in carriers with this pattern than in carriers who were HBsAg positive but anti-HBs negative (P=0.046 and P<0.001, respectively).

Our findings were similar to a study by Liu et al.,[15] which showed a 2.9% prevalence of the coexistence of HBsAg and anti-HBs in Chinese CHB patients. There was no significant difference between patients with and without anti-HBs regarding age, gender, alanine aminotransferase level, and the proportion positive for HBeAg and HBcAb.

The present findings found that 2.8% (2/70) of patients were positive for the coexistence of HBeAg and HBeAb, which was closely similar to the study by Rabbie et al. in Dhaka,[27] in which out of 72 chronic HBsAg positive carriers, 28(38.9%) patients were HBeAg positive and anti-HBe negative, 38(52.8%) patients were HBeAg negative and anti-HBe positive, only 3(4.2%) patients were positive for both HBeAg and anti-HBe and the rest 3(4.2%) patients were negative for both markers.

In a study by Xiang et al.,[29] among 124,865 patients with CHB infection, 324(0.3%) were concurrently positive for HBsAg and anti-HBs, and 206(0.2%) were concurrently positive for HBeAg and anti-HBe. The HBeAg+/anti-HBe+ group was composed of younger patients (P<0.05). There were no significant difference in the sex, ALT, AST, and HBV DNA level between the HBsAg+/anti-HBs+ and the HBeAg+/anti-HBe+ groups. In our study, the percentage of positive results for the HBeAg/HBeAb coexistence was higher among the patients between 19-40 years than among the older group, but with an insignificant difference. This result contrasts with a study by Xiang et al.[29] This current study revealed no substantial relationship between the coexistence of HBsAg/HBsAb and HBeAg/HBeAb with gender, age and treatment, residence, and the incidence of anti-HBe.

Conclusion

The frequencies of the coexistence of HBsAg/HBsAb and HBeAg/HBeAb among Sudanese patients with chronic HBV infection are low compared to previous studies in a different population.

Competing Interests

The authors declare that they have no competing interests.

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International Journal of Biomedicine 13(4) (2023) 286-295
http://dx.doi.org/10.21103/Article13(4)_OA9

ORIGINAL ARTICLE

COVID-19

INTERNATIONAL JOURNAL OF BIOMEDICINE

The Association of Prolactin and CRP Biomarkers with the Severity of COVID-19 in Thumbay Hospital, Ajman, UAE

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Abstract

Background: This study aimed to estimate the levels of C-reactive protein (CRP) and prolactin (PRL) in SARS-CoV2 infection and their association with the severity of COVID-19 among patients in Ajman, UAE.

Methods and Results: This cross-sectional study was conducted in Thumbay Hospital from 2020 to 2021. The study included 71 patients (55 males and 16 females) with positive SARS-CoV-2 test results. Nasal swab specimens were collected for the COVID-19 test on the day of admission or after one day of admission. COVID-19 diagnoses and severity levels were determined according to the New Coronavirus Pneumonia Prevention and Control Program (7th edition) published by the National Health Commission of China (2020).

Serum samples were collected from the patients upon admission. The PRL level was determined using the immune chemiluminescent method by the DXI 800 Beckman Coulter analyzer. The CRP level was determined using the immunoturbidimetric method by the DXC 700 AU chemistry analyzer.

Among 71 COVID-19 patients, the great majority were men 55(77.5%), 38(53.5%) being of Indian nationality. In this study, most participants (50.7%) had no history of chronic illnesses. In terms of COVID-19 severity, 24(33.8%) of patients had mild cases, 27(38.0%) had moderate cases, and 20(28.2%) had severe cases. Twenty (28.2%) patients were transferred to the ICU, and 19(26.8%) were intubated. The patients' average age was 47.58±13.63, CRP level - 74.30±71.46 mg/L, and PRL level - 205.1946±168.52 ng/mL. The mean CRP level was highest in severe cases, compared to mild and moderate cases, with a statistically significant difference between mild and severe groups (P=0.000) and mild and moderate groups (P=0.004). The mean PRL level was highest in severe cases compared to mild and moderate cases; however, the differences between the groups were not significant. CRP and PRL levels were greater in the ICU patients than non-ICU patients, with statistically significant differences only for CRP. We found a moderate positive correlation between CRP level and age (r=0.458, P=0.000); a weak positive correlation between PRL level and age was not statistically significant (r=0.201, P=0.093). A moderate positive correlation between CRP level and PRL level (r=0.461, P=0.03) was statistically significant.

Conclusion: The current study implies that serum CRP levels might be an important indication of COVID-19 development and severity. A more extensive study with a larger sample size is needed to validate the significance of PRL in disease severity. **(International Journal of Biomedicine. 2023;13(4):286-295.)**

Keywords: COVID-19 • prolactin • C-reactive protein

For citation: Nur AA, Osman AL, Kandakurti PK, Alfeel AA, Ismail M, Babker AMa, Altoum AA. The Association of Prolactin and CRP Biomarkers with the Severity of COVID-19 in Thumbay Hospital, Ajman, UAE. International Journal of Biomedicine. 2023;13(4):286-295. doi:10.21103/Article13(4)_OA9

Abbreviations

BP, blood pressure; **BMI**, body mass index; **CRP**, C-reactive protein; **COVID-19**, coronavirus disease 2019; **DM**, diabetes mellitus; **GH**, growth hormone; **hs-CRP**, high-sensitivity CRP; **HPT**, Hypertension; **ICU**, intensive care unit; **LH**, luteinizing hormone; **PRL**, prolactin; **SARS-CoV-2**, severe acute respiratory syndrome coronavirus-2.

Introduction

Coronaviruses (CoVs), enveloped positive-strand RNA viruses from the *Coronaviridae* family, are associated with upper respiratory tract infections that occasionally spread to the lungs and other organs. As well as the common cold, CoVs can cause more severe diseases, including SARS-CoV disease, MERS-CoV, and COVID-19, which was caused by SARS-CoV-2. As of 20 August 2023, over 769 million confirmed cases of COVID-19 and over 6.9 million deaths have been reported globally[1] Typical manifestations include flu-like symptoms such as fever, cough, fatigue, and shortness of breath. However, in approximately 20% of patients, the infection progresses to severe interstitial pneumonia and can cause an uncontrolled host immune response, leading to a life-threatening condition called cytokine storm;[2] it is critical to identify early and treat this subgroup of patients. The degree of severity and mortality of patients with COVID-19 may be associated with altered levels of some blood markers.

One such marker is C-reactive protein (CRP). It is one of the clinical parameters that indicates more severe infection and has been used as an indicator of COVID-19 disease severity.[3] CRP is a plasma protein produced by the liver and induced by various inflammatory mediators, such as IL-6. Clinically, CRP is used as a biomarker for various inflammatory conditions, so any rise in its level indicates an increased disease severity but not specific to a particular disease.[4] CRP has been recognized as one of the most, if not the most, sensitive acute phase reactants. CRP levels in plasma can rise dramatically after myocardial infarction, stress, trauma, infection, or neoplastic proliferation. The CRP marker was found to be significantly increased in the initial phases of the infection for severe COVID-19 patients. Importantly, CRP has been associated with disease development and is an early predictor for severe COVID-19.[5] There are two tests that measure CRP, and each test measures a different range of CRP levels in the blood for different purposes: The standard CRP test measures markedly high protein levels to detect diseases that cause significant inflammation. It measures CRP in the range from 10 to 1000 mg/L. This test may be used to detect inflammation. However, hs-CRP test is more sensitive than a standard CRP test. The hs-CRP test accurately detects lower protein levels than the standard CRP test. It measures CRP in the range from 0.5 to 10mg/L. This test is used to evaluate individuals for the risk of cardiovascular disease. Many studies have indicated that CRP levels can help predict the severity and presence of COVID-19 infections.[6]

Hormonal homeostasis has a major impact on achieving competent and healthy immune system function. Prolactin (PRL) has a bioactive function, acting as a hormone and cytokine.[7] Hyperprolactinemia has been detected in many patients with different autoimmune diseases, such as rheumatoid arthritis, systemic lupus erythematosus, Sjögren syndrome, multiple sclerosis, autoimmune thyroid disease, systemic sclerosis, and others. High PRL levels have been shown in patients with severe sepsis and infants with severe respiratory infections. At the same time, long-term hypoprolactinemia can cause death from opportunistic infections in patients with HIV infection.[8] PRL has both pro-inflammatory and anti-inflammatory roles, depending on certain conditions.[9-12]

High blood PRL level is known to provide an immunological advantage in many pathological conditions (with some exceptions like autoimmune diseases), and women, because of their higher blood PRL level, get an advantage in this regard. It has been reported that enhancement of PRL serum level toward the physiological values by dopamine antagonists may improve the immunological profile and survival in various critical statuses.[13,14] PRL acts through specific PRL receptors (PRLRs) belonging to the class I cytokine receptor family, which includes more than 30 receptors, such as the PRLR, GH receptor, thrombopoietin receptor, IL-6 receptor, and others.[15] PRLRs can also act as function receptors for GH and placental lactogen.[16] Also, PRL may serve as a cytokine-like action via the activation of cytokine receptors in the regulation of the immune system.[17]) PRL stimulates T and B cells, natural killer cells, macrophages, neutrophils, CD34+ hematopoietic cells, and antigen-presenting dendritic cells.[18] It influences the modulation of the immune system, mainly by inhibiting the negative selection of autoreactive B lymphocytes.[7]

SARS-CoV-2 infection can impair the hypothalamic–pituitary–gonadal axis and, by this mechanism, may increase the secretion of PRL from the anterior pituitary in COVID-19.[19] The underlying mechanisms of high serum PRL levels in COVID-19 are poorly understood. However, stress and immune dysregulation may be potential mechanisms, as stressful conditions in COVID-19 may trigger PRL release.[20-22] The reduction in brain dopamine levels by SARS-CoV-2 may remove the inhibitory effects on prolactinemia, leading to the hyperprolactinemia observed in patients with COVID-19. In addition, high pro-inflammatory cytokine levels, including IL-6, in COVID-19 are considered a potent stimulator of PRL from the anterior pituitary gland.[23,24] High arginine-vasopressin and angiotensin II serum levels during COVID-19 might be another mechanism for COVID-19-induced hyperprolactinemia.[25]

In general, PRL production is increased in SARS-CoV2 infection. While PRL can trigger the production of proinflammatory cytokines, it also has several anti-inflammatory effects[14] that can reduce hyperinflammation. The exact mechanism of PRL's contribution to the severity of COVID-19 is unknown.

This study aimed to estimate the levels of CRP and PRL in SARS-CoV2 infection and their association with the severity of COVID-19 among patients in Ajman, UAE.

Materials and Methods

This cross-sectional study was conducted in Thumbay Hospital from 2020 to 2021. The study included 71 patients (55 males and 16 females) with positive SARS-CoV-2 test results. The information for all patients, including demographic data, clinical characteristics, laboratory parameters, and outcomes, was collected prospectively. Nasal swab specimens were collected for the COVID-19 test on the day of admission or after one day of admission. RT-PCR assay was used as per the manufacturer's instructions.

Serum samples were collected from the patients upon admission. The PRL level was determined using the immune chemiluminescent method by the DXI 800 Beckman Coulter analyzer. Hyperprolactinemia was defined when fasting PRL serum levels were more than 25 ng/mL in females and 20 ng/mL in males.[26] The CRP level was determined using the immunoturbidimetric method by the DXC 700 AU chemistry analyzer. Normal CRP levels were defined as below 3.0 mg/L.[27]

The validation procedure was done according to CAP and CLIA for precision, accuracy, and linearity.

COVID-19 diagnoses and severity levels were determined according to the New Coronavirus Pneumonia Prevention and Control Program (7th edition) published by the National Health Commission of China.[28] Briefly, mild disease was defined as mild symptoms without radiographic features. Moderate disease was defined as fever, respiratory symptoms, and radiographic features. Severe cases met one of the following three criteria: (a) dyspnea, with a respiratory rate ≥ 30 times/min, (b) oxygen saturation ≤ 93% at rest, or (c) PaO_2/FiO_2 ≤ 300mmHg. Critical cases met one of the following three criteria: (a) respiratory failure, (b) septic shock, or (c) multiorgan failure.

In this study, we defined the moderate group as the mild and moderate cases, and we defined the severe group as the severe and critical cases. The duration of viral shedding was defined as the time from symptom onset to a SARS-CoV-2 RNA throat swab turning negative. 'Long-term positive' cases were defined as cases with a duration of viral shedding > 50 days, and the other cases were assigned to the normal-term group. Data was stored and retrieved from the laboratory information system (Thumbay Labs, Ajman, UAE).

Statistical analysis was performed using the statistical software package SPSS version 26.0 (SPSS Inc, Armonk, NY: IBM Corp). For the descriptive analysis, results are presented as mean (M) ± standard deviation (SD). The two-sample z-test and independent t-test were used to test the difference between the means of two groups, whereas a one-sample z-test was used to test the difference between a single group and the hypothesized population value. Multiple comparisons were performed with one-way ANOVA and Tukey HSD post-hoc test. Pearson's correlation coefficient (r) was used to determine the strength of the relationship between the two continuous variables. A value of $P<0.05$ was considered significant.

Results

Among 71 COVID-19 patients, the great majority were men 55(77.5%), 38(53.5%) being of Indian nationality. In this study, most participants (50.7%) had no history of chronic illnesses. In terms of COVID-19 severity, 24(33.8%) of patients had mild cases, 27(38.0%) had moderate cases, and 20(28.2%) had severe cases. Twenty (28.2%) patients were transferred to the ICU, and 19(26.8%) were intubated.

The disease's signs and symptoms are presented in Table 1. The patients' average age was 47.58±13.63, CRP level - 74.30±71.46 mg/L, and PRL level - 205.1946±168.52 ng/mL (Table 2).

Table 1.
Baseline characteristics of the study group.

Variables	Group	Frequency	Percentage
Gender	Males	55	77.5%
	Females	16	22.5%
Nationality	Arab	17	23.9%
	Indian	38	53.5%
	Pakistani	7	9.9%
	Other	9	12.7%
Comorbidities	No Comorbidities	36	50.7%
	Diabetes	10	14.1%
	HPT	6	8.5%
	HPT & DM	11	15.5%
	Other	8	11.3%
Severity	Mild	24	33.8%
	Moderate	27	38.0%
	Severe	20	28.2%
ICU admission	Yes	20	28.2%
	No	51	71.8%
Mechanical Ventilator (Intubation)	Yes	19	26.8%
	No	52	73.2%
Patients' outcome	Alive	62	87.3%
	Dead	9	12.7%
Descriptive Statistics of Patients Symptoms			
Headache	Yes	30	42.3%
	No	41	57.7%
Fever	Yes	67	94.4%
	No	4	5.6%
Cough	Yes	66	93.0%
	No	5	7.0%
Fatigue	Yes	52	73.2%
	No	19	26.8%
Pneumonia	Yes	22	31.0%
	No	49	69.0%
Shortness of breath	Yes	40	56.3%
	No	31	43.7%

The mean CRP level was highest in severe cases (122.895±76.3050 mg/L), compared to mild (25.154±25.6865 mg/L) and moderate cases (81.993±69.1761 mg/L) (Table 3), with a statistically significant difference between mild and severe groups (P=0.000) and mild and moderate groups (P=0.004) (Table 4 and Table 5).

Table 2.

Characteristics of clinical parameters.

Parameters	N	Minimum	Maximum	Mean	SD
Age, yrs.	71	24	78	47.58	13.629
BMI, kg/m^2	71	15.70	41.02	28.0906	4.47115
Oxygen saturation, %	71	70.00	99.00	94.1972	5.05009
Respiratory rate, breaths per minute	71	18	44	24.93	5.680
Temperature, °C	71	36.5	39.0	37.794	0.6618
Diastolic BP, mmHg	71	43	100	73.87	10.105
Systolic BP, mmHg	71	80	179	120.79	15.059
CRP, mg/L	71	5.0	278.2	74.301	71.4158
White blood count, $\times10^9$/L	71	1.8	22.8	8.338	4.0439
Neutrophils, $\times10^9$/L	71	0.68	21.73	6.5023	4.14271
Lymphocytes, $\times10^9$/L	70	0.11	4.50	1.1789	0.74152
PRL, ng/mL	71	57.68	1218.45	205.1946	168.52532

Table 3.

CRP level and COVID-19 severity.

COVID-19 severity	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Min	Max
					Lower Bound	Upper Bound		
Mild	24	25.154	25.6865	5.2432	14.308	36.001	5.0	84.5
Moderate	27	81.993	69.1761	13.3129	54.627	109.358	6.0	249.5
Severe	20	122.895	76.3050	17.0623	87.183	158.607	18.6	278.2
Total	71	74.301	71.4158	8.4755	57.398	91.205	5.0	278.2

Table 4.

Analysis of variance (ANOVA) of CRP level with COVID-19 severity.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	106794.742	2	53397.371	14.511	0.000
Within Groups	250220.628	68	3679.715		
Total	357015.370	70			

Table 5.

Multiple Comparisons (Post-Hoc) of CRP level with COVID-19 severity.

Dependent Variable: CRP						
(I) Severity	(J) Severity	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Mild	Moderate	-56.8384	17.0178	0.004	-98.611	-15.066
	Severe	-97.7408	18.3659	0.000	-142.823	-52.659
Moderate	Mild	56.8384	17.0178	0.004	15.066	98.611
	Severe	-40.9024	17.8961	0.076	-84.831	3.026
Severe	Mild	97.7408	18.3659	0.000	52.659	142.823
	Moderate	40.9024	17.8961	0.076	-3.026	84.831

The mean PRL level was highest in severe cases (255.975±263.56 ng/mL) compared to mild (187.990±104.361 ng/mL) and moderate cases (182.8726±115.28912 ng/mL) (Table 6). However, the differences between the groups were not significant (Table 7 and Table 8).

Table 6.

Prolactin level and COVID-19 severity.

COVID-19 severity	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Min	Max
					Lower Bound	Upper Bound		
Mild	24	187.9900	104.36156	21.30272	143.9220	232.0580	71.40	527.96
Moderate	27	182.8726	115.28912	22.18740	137.2657	228.4795	57.68	553.49
Severe	20	255.9750	263.56291	58.93446	132.6238	379.3262	62.80	1218.45
Total	71	205.1946	168.52532	20.00028	165.3054	245.0839	57.68	1218.45

CRP and PRL levels were greater in the ICU patients than non-ICU patients (Table 9), with statistically significant differences only for CRP (Table 10). No statistically significant difference in PRL and CRP levels with gender was found (Tables 11 and 12).

We found a moderate positive correlation between CRP level and age (r=0.458, *P*=0.000) (Table 13, Figure 1); a weak positive correlation between PRL level and age was not statistically significant (r=0.201, *P*>0.093) (Table 14, Figure 2).

Table 7.

Analysis of variance (ANOVA) of Prolactin level with COVID-19 severity.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	72130.283	2	36065.142	1.280	0.285
Within Groups	1915924.563	68	28175.361		
Total	1988054.846	70			

Table 8.

Multiple Comparisons (Post-Hoc) of Prolactin level with COVID-19 severity.

Dependent Variable: prolactin						
(I) Severity	(J) Severity	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Mild	Moderate	5.11741	47.09039	1.000	-110.4728	120.7076
	Severe	-67.98500	50.82068	0.556	-192.7317	56.7617
Moderate	Mild	-5.11741	47.09039	1.000	-120.7076	110.4728
	Severe	-73.10241	49.52070	0.434	-194.6582	48.4533
Severe	Mild	67.98500	50.82068	0.556	-56.7617	192.7317
	Moderate	73.10241	49.52070	0.434	-48.4533	194.6582

Table 9.

Group statistics of CRP and Prolactin with ICU admission.

	ICU Admission	N	Mean	Std. Deviation	Std. Error Mean
CRP	Yes	20	123.295	71.0223	15.8811
	No	51	55.088	62.3446	8.7300
PRL	Yes	20	254.4780	263.95167	59.02139
	No	51	185.8678	109.24183	15.29692

Table 11.

Group statistics of CRP and Prolactin with gender.

	Gender	N	Mean	Std. Deviation	Std. Error Mean
CRP	Male	55	79.260	70.2936	9.4784
	Female	16	57.256	74.9177	18.7294
PRL	Male	55	194.3769	174.86898	23.57933
	Female	16	242.3806	143.34554	35.83638

Table 10.

Independent samples t-test of CRP and Prolactin with ICU admission.

		Levene's Test for equality of variances		t-test for equality of means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
CRP	Equal variances assumed	0.868	0.355	3.98	69	0.000	68.2068	17.1096	34.0741	102.3394
	Equal variances not assumed			3.76	31.1	0.001	68.2068	18.1224	31.2525	105.1610
PRL	Equal variances assumed	7.82	0.007	1.55	69	0.124	68.6101	44.0152	- 19.1979	156.4182
	Equal variances not assumed			1.12	21.6	0.273	68.6101	60.9714	- 57.9724	195.1927

Table 12.

Independent samples t-test of CRP and PRL with gender.

		Levene's Test for equality of variances		t-test for equality of means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
CRP	Equal variances assumed	0.069	. 0.794	1.08	69	0.281	22.0038	20.2594	- 18.412	62.4201
	Equal variances not assumed			1.04	23.2	0.305	22.0038	20.9912	- 21.394	65.4022
PRL	Equal variances assumed	0.002	0.961	-1.0	69	0.319	- 48.003	47.8668	- 143.49	47.4881
	Equal variances not assumed			-1.1	29.2	0.272	- 48.003	42.8979	- 135.70	39.6965

Table 13.

Pearson correlation between CRP and age.

		CRP	Age
CRP	Pearson correlation	1	0.458
	Sig. (2-tailed)		0.000
	N	71	71
Age	Pearson correlation	0.458	1
	Sig. (2-tailed)	0.000	
	N	71	71

Table 14.

Pearson correlation between PRL and age.

		Age	Prolactin
Age	Pearson correlation	1	0.201
	Sig. (2-tailed)		0.093
	N	71	71
PRL	Pearson Correlation	0.201	1
	Sig. (2-tailed)	0.093	
	N	71	71

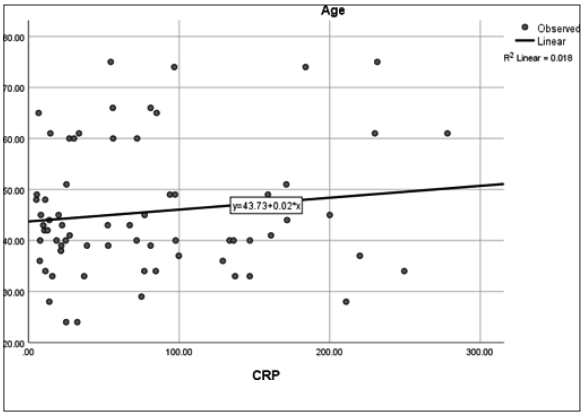

Fig. 1. *Scatterplot: Relationship between CRP and age.*

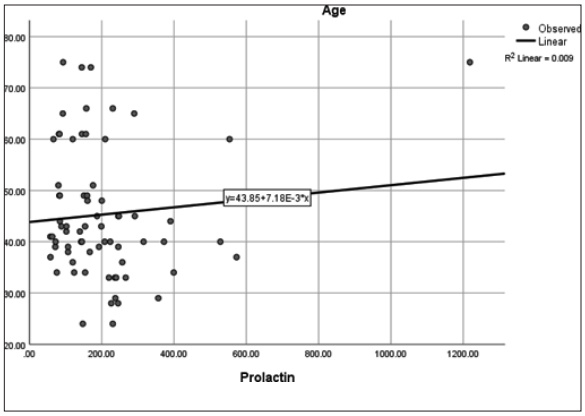

Fig. 2. *Scatterplot: Relationship between PRL and age.*

We found a moderate positive correlation between CRP level and PRL level age (r=0.461, *P*=0.03) (Table 15, Figure 3).

Table 15.

Pearson correlation between CRP and PRL.

		CRP	PRL
CRP	Pearson correlation	1	0.461
	Sig. (2-tailed)		0.03
	N	71	71
PRL	Pearson correlation	0.461	1
	Sig. (2-tailed)	0.03	
	N	71	71

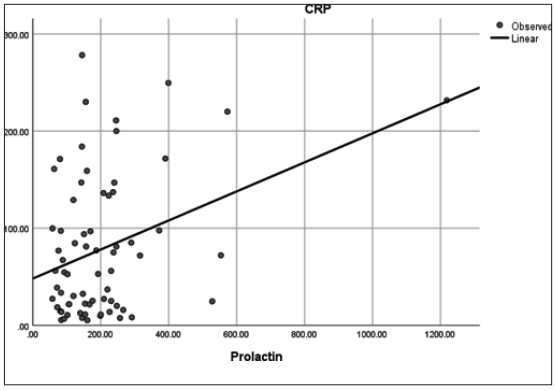

Fig. 3. *Scatterplot: Relationship between PRL and CRP.*

Discussion

The current research found that severe cases of COVID-19 had considerably higher CRP levels than mild cases, and moderate cases had considerably higher CRP levels than mild cases. However, the results revealed that the PRL level was not associated with the severity of the condition.

This study differs from other studies in the field due to the different races and nationalities. The country of the United Arab Emirates has a culture of variety. Other studies were carried out in a homogeneous nationality. The effect of PRL on COVID-19 severity has not been thoroughly researched. As indicated in the analysis performed above, the severity of COVID-19 disease was not associated with PRL levels. Similarly, another study found that infected patients with COVID-19 have similar susceptibility to infection and disease severity irrespective of their PRL levels.[29] In contrast, Zare-Zardini et al.[30] performed a study to determine the influence of PRL on COVID-19 infection susceptibility and severity. According to the study, lower blood PRL levels in patients enhance their susceptibility to and severity of COVID-19 infection. The low frequency of COVID-19 among children and women has been attributed to various molecular and physiological factors.[30,31] Although pregnant women have high PRL levels, they are more susceptible to COVID-19 than the general population.[32] Other studies reported that pregnant and non-pregnant women have similar susceptibility to COVID-19.[33] However, higher PRL levels during pregnancy may give pregnant women an advantage in fighting COVID-19. Thus, in a study by Liu et al.,[34] all cases of COVID-19 pneumonia in pregnant women were mild. While men and women have the same prevalence, men with COVID-19 are more at risk for worse outcomes and death, independent of age.[35] It is known that women have higher blood PRL levels than men, and their levels do not fall after menopause.[36] Yamaji et al.[37] demonstrated that PRL secretion is enhanced in female subjects throughout life after puberty and that aging, per se, is not associated with an alteration in the rate of secretion of this hormone in human subjects. Therefore, higher PRL levels in women may explain the gender difference in COVID-19. Early studies suggested that cigarette smokers were more resistant to coronavirus, possibly due to nicotine's ability to increase serum PRL levels.[38]

Many COVID-19 patients in this research had increased CRP levels, consistent with findings from prior investigations. Furthermore, severe cases in this research had considerably higher CRP levels than mild and moderate cases, suggesting that CRP might be a serum marker for severe COVID-19 patients. The current findings are consistent with those of Chen et al.,[39] who demonstrated that the plasma CRP level was positively correlated to the severity of COVID-19 pneumonia.

The close positive association between CRP values and the severity of tissue damage in many different pathologies, notably including COVID-19, illustrated by Smilowitz et al.[40] in the exemplary study, is equally consistent with a pathogenic role for CRP. CRP associated with tissues damaged by the virus and/or the host response locally activates complement, guaranteeing aggravation of the damage, as well as promoting systemic activation of complement. A new small molecule drug that inhibits CRP binding in vivo is currently being developed to test whether this CRP-complement mechanism contributes significantly to the severity of COVID-19.[41]

A large cohort study of 1275 COVID-19 patients showed that high CRP and D-dimer levels at admission (≥150 mg/L and ≥1000 ng/ml, respectively) and a peak D-dimer ≥6000 ng/ml during hospital stay were independent factors associated with pulmonary embolism.[42] Along with high levels of CRP, the progressive increase in leukocyte count and sustained lymphopenia and eosinopenia in severe COVID-19 patients may be associated with the progression of inflammatory status, which might progress to a fatal clinical outcome.[43,44]

Accumulating evidence has indicated that CRP is not only an excellent biomarker of inflammation but also acts as a direct participant in the SARS-CoV-2 infection.[45] Several studies have proposed CRP as a marker of cytokine

storm in COVID-19 patients.[46,47] Moreover, increased CRP levels in the blood can be detected in the earliest stages of the disease,[47-50] even before lung lesions.[46,51] Thus, CRP represents a very useful tool for identifying patients in need of immediate attention and closer clinical follow-up.[52] CRP levels are not only an early marker of disease stratification, but also a valuable tool for predicting the development of COVID-19.[46,51] Today, it is generally accepted that COVID-19 can manifest itself in different ways, from mild to critical conditions leading to death. In this context, the discovery of reliable biomarkers and therapeutic targets merits further targeted research.[52] CRP and serum ferritin levels might be considered as an essential indication of the progression and severity of COVID-19.[53]

Limitations

There are several limitations to this study. First, it is single-center retrospective research with a limited sample size, which limits the data's generalizability. Second, missing data was discovered in many cases. Third, only hospitalized patients were documented. Children and pregnant women should be included in future studies to examine their PRL levels and compare them to the general population.

Conclusion

The current study implies that serum CRP levels might be an important indication of COVID-19 development and severity. A more extensive study with a larger sample size is needed to validate the significance of PRL in disease severity.

Ethical Considerations

The study protocol was reviewed and approved by the Ethics Committee of the Gulf Medical University, Ajman, UAE**.** Written informed consent was obtained from all the participants.

Competing Interests

The authors declare that they have no competing interests.

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International Journal of Biomedicine 13(4) (2023) 296-300
http://dx.doi.org/10.21103/Article13(4)_OA10

ORIGINAL ARTICLE

COVID-19

INTERNATIONAL JOURNAL OF BIOMEDICINE

Kidney Injury Incidence in COVID-19 Patients and Evaluation of Several Function Variables

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Abstract

Background: The clinical spectrum of SARS-CoV-2 infection includes conditions from asymptomatic and mild-moderate respiratory illnesses to catastrophic viral pneumonia with respiratory failure, septic shock, and multiple organ dysfunction. The aim of this study was to assess the prevalence of acute kidney injury (AKI) in COVID-19 patients treated at AL-Saddar Teaching Hospital (Najaf, Iraq).

Methods and Results: The study population consisted of 190 patients treated at AL-Saddar Teaching Hospital (Najaf, Iraq) between June 1 and August 20, 2022. Clinical signs, lung abnormalities, and a positive result from real-time PCR for nasopharyngeal swab samples have all been used to identify infection. Clinical and laboratory information on the patients was gathered for the investigation. A comparative analysis was conducted between patients with AKI (n=67) and without AKI (n=123). The patients without AKI frequently left the hospital in better health (80.9%), and they did not require dialysis, compared to 22.4% of those who had AKI. The percentage of patients in each group admitted to the intensive care unit (ICU) significantly differed with respect to KI: 46.3% with AKI and 15.4% without AKI (P<0.0001). Furthermore, patients with AKI had a higher rate of mortality (13.4%) than those without AKI (0.8%) (P=0.0002).

Conclusion: The results indicated that AKI is prevalent in hospitalized COVID-19 patients and is attributed to in-hospital death rates.**(International Journal of Biomedicine. 2023;13(4):296-300.)**

Keywords: COVID-19 • kidney injury • intensive care unit

For citation: Salman KA. Kidney Injury Incidence in COVID-19 Patients and Evaluation of Several Function Variables.. International Journal of Biomedicine. 2023;13(4):296-300. doi:10.21103/Article13(4)_OA10

Abbreviations

AKI, acute kidney injury; **COVID-19**, coronavirus disease 2019; **CKD**, chronic kidney disease; **CRP**, C-reactive protein; **ICU**, intensive care unit; **SARS-CoV-2**, severe acute respiratory syndrome coronavirus-2.

Introduction

Globally, the coronavirus disease COVID-19 caused by SARS-CoV-2 represents a severe public health crisis. The clinical spectrum of SARS-CoV-2 infection includes conditions from asymptomatic and mild-moderate respiratory illnesses to catastrophic viral pneumonia with respiratory failure, septic shock, and multiple organ dysfunction.[1] The likelihood of a severe clinical presentation of COVID-19 is increased by advanced age, immunosuppressive medications, and underlying comorbidities such as cancer, diabetes, chronic kidney disease (CKD), cardiovascular diseases, diabetes, and chronic pulmonary illnesses.[2] The definite mechanism by which the virus enters host cells is the protein angiotensin-converting enzyme 2 (ACE2), which is abundantly present in the lungs.[3] It is important to note that acute kidney damage, proteinuria, and hematuria have all been independently associated with a higher risk of dying in COVID-19 individuals.[4,2] The steady decline in renal function in CKD alters the innate and adaptive immune systems. The loss of B lymphocytes, rapid T cell turnover, and a rise in CD4+ and

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CD8+ lymphocyte death are among these alterations, as well as a decrease in the number of dendritic cells that transmit antigens.[5] Infections account for a significant portion of mortality, particularly in patients with end-stage renal illness, and the weakened immune response is linked to increased incidence and more severe courses of infections. In addition to secondary immunodeficiency, immunological activation is seen in patients with chronic renal disease.[6] Atherosclerotic cardiovascular disease and other problems that impair the prognosis of patients with SARS-CoV-2 infection are brought on by the increased synthesis and poor clearance of pro-inflammatory cytokines, which cause systemic inflammation and oxidative stress.[7]

According to research from China, acute kidney injury (AKI) has been documented in 29% of COVID-19 patients who are severely sick or have passed away.[8] It is significant to highlight that AKI, proteinuria, and hematuria have all been independently linked to a greater risk of passing away in COVID-19 patients.[9] In addition, in a meta-analysis involving 1389 COVID-19 patients, individuals with a severe COVID-19 condition had a considerably higher frequency of underlying CKD (3.3% vs. 0.4%).[10]

The first laboratory-confirmed case of COVID-19 in Iraq was reported on February 21, 2020, and it was an Iranian citizen who entered the country before the decision to prevent travel services. Afterwards, the infection cases gradually declined. However, on June 20, 2022, and through the follow-up of the epidemiological monitoring teams of the Ministry of Health to the latest developments in the daily epidemiological situation of the coronavirus pandemic in Iraq, it was noted that there was a noticeable increase in the percentage of positive cases from total examinations, as well as an increase in the number of hospitalized cases, which means that Iraq had entered a new epidemic wave.

The aim of this study was to assess the prevalence of acute kidney injury (AKI) in COVID-19 patients treated at AL-Saddar Teaching Hospital (Najaf, Iraq)

Materials and Methods

The study population consisted of 190 patients treated at AL-Saddar Teaching Hospital (Najaf, Iraq) between June 1 and August 20, 2022.

Clinical signs, lung abnormalities, and a positive result from real-time PCR for nasopharyngeal swab samples have all been used to identify infection. Clinical and laboratory information on the patients was gathered for the investigation. The following information was gathered: age, gender, comorbidities, complete blood count, and readings for glucose, urea, creatinine, CRP, ferritin, and the CT results. A computerized chemistry analyzer and ready-to-use reagent kits were used for all biochemical assays, which were completed according to the instructions provided by the manufacturers (Mindray BS 2000M, China). Medical record system data was gathered to ensure patients' privacy, and an anonymous analysis was performed.

Statistical analysis was performed using the statistical software package SPSS version 21.0 (SPSS Inc, Armonk, NY: IBM Corp). Baseline characteristics were summarized as frequencies and percentages for categorical variables and as mean (Min-Max) for continuous variables. Group comparisons were performed using chi-square tests or, alternatively, Fisher's exact test (2-Tail) when expected cell counts were less than 5. A probability value of $P<0.05$ was considered statistically significant.

Results

Between June 1st and August 20th, 2022 (the new wave of the epidemic), 190 patients admitted to AL-Saddar Teaching Hospital/Najaf's central laboratory had a COVID-19 test performed or a test had been done previously. The clinical features of patients with COVID-19 are described in the tables below. A comparative analysis was conducted between patients with AKI (n=67) and without AKI (n=123). Table 1 showed that the patients without AKI frequently left the hospital in better health (80.9%), and they did not require dialysis, compared to 22.4% of those who had AKI. The percentage of patients in each group admitted to the intensive care unit (ICU) significantly differed with respect to AKI: 46.3% with AKI and 15.4% without AKI ($P<0.0001$). Furthermore, patients with AKI had a higher rate of mortality (13.4%) than those without AKI (0.8%) ($P=0.0002$) (Table 1).

Table 1.

Clinical features of COVID-19 patients with and without AKI.

Variables	COVID-19 patients without AKI (n=123)	COVID-19 patients with AKI (n=67)	*P*-value
	n (%)	n (%)	
Admission to the ICU	19 (15.4)	31 (46.3)	<0.0001
Dialysis	2 (1.6)	15 (22.4)	<0.0001
Recovery cases	102 (82.9)	12 (17.9)	<0.0001
Mortality	1 (0.8%)	9 (13.4)	0.0002

The manifestations of COVID-19 patients without AKI were fever (48.8%), cough (52.0%), sore throat (43.9%), labored breathing (9.8%), diarrhea (4.9%), fatigue (14.6%), and abdominal pain (3.2%). The manifestations of COVID-19 patients with different AKI rates depended on the KI intensity (Table 2). In this study, patients who developed KI had a significant relationship with hypertension (56.7%), diabetes (38.8%), chronic liver disease (23.9%), asthma (16.4%), and cancer (13.4%) (Table 3). We found the highest levels of inflammatory markers and kidney function tests in patients with AKI, including D-dimer (1944 vs. 501 mg/ml), C-reactive protein (890 vs. 358.5 mg/dl), serum creatinine (8 vs. 1.6 mg/dl), and blood urea (175.5 vs. 32.5 mg/dl), compared to patients without kidney injury (Table 4). Regarding the age groups and gender, we did not find noticeable differences by different AKI severity (Table 5)

Table 2.

Clinical symptoms considering the severity of AKI.

Symptoms	COVID-19 patients without AKI (n=123)	COVID-19 patients with AKI (n=67)		
	n (%)	Mild (n=25)	Moderate (n=22)	Severe (n=20)
		n (%)	n (%)	n (%)
Fever	60 (48.8)	18 (72.0)	18 (81.8)	13 (65.0)
		$\chi^2 = 1.534$; *P*=0.464		
Cough	64 (52.0)	21 (84.0)	19 (86.4)	18 (90.0)
		$\chi^2 = 0.345$; *P*=0.842		
Sore throat	54 (43.9)	16 (64.0)	11 (50.0)	15 (75.0)
		$\chi^2 = 2.829$; *P*=0.243		
Labored breathing	12 (9.8)	4 (16.0)	3 (13.6)	19 (95.0)
		$\chi^2 = 37.94$; *P*=0.000		
Diarrhea	6 (4.9)	1 (4.0)	1 (4.5)	4 (20.0)
		$\chi^2 = 4.17$; *P*=0.118		
Fatigue	18 (14.6)	5 (20.0)	4 (18.2)	8 (40.0)
		$\chi^2 = 3.242$; *P*=0.198		
Abdominal pain	4 (3.2)	1 (4.0)	1 (4.5)	2 (10.0)
		$\chi^2 = 0.831$; *P*=0.660		

Table 4.

Inflammatory markers in COVID-19 patients with and without AKI.

Laboratory parameters	COVID-19 patients without AKI (n=123) Mean (Min-Max)	COVID-19 patients with AKI (n=67) Mean (Min-Max)
CRP, mg/dL	358.5 (66–585)	890 (500–1280)
Blood urea, mg/dL	32.5 (18–47)	175.5 (68–215)
Serum creatinine, mg/dL	1.6 (0.9–2.2)	8 (1.8–14.1)
D-dimer, mg/mL	501 (224–778)	1944 (889–2999)
Hb, g/dL	13.2 (12.5–13.9)	11.7 (10.5–12.9)
WBCs, $\times 10^3/\mu$ L	7.2 (5.4–8.9)	10.5 (5.9–15.1)

Table 3.

The frequency of comorbidities in COVID-19 patients with and without AKI.

Comorbidities	COVID-19 patients without AKI (n=123)	COVID-19 patients with AKI (n=67)	*P*-value
	n (%)	n (%)	
Cardiac disease	28 (22.8)	19 (28.4)	0.3942
Diabetes	22 (17,9)	26 (38.8)	0.0016
Hypertension	30 (24.4)	38 (56.7)	<0.0001
Chronic liver disease	12 (9.8)	16 (23.9)	0.0090
Asthma	7 (5.7)	11 (16.4)	0.0164
Cancer	3 (2.4)	9 (13.4)	0.0029
No known chronic diseases	33 (26.8)	24 (35.8)	0.1969

Table 5.

The severity of AKI regarding the age and gender of COVID-19 patients

Age, years	COVID-19 patients with AKI (n=67)									
	Mild			Moderate			Severe			
	Female n (%)	Male n (%)	Total n (%)	Female n (%)	Male n (%)	Total n (%)	Female n (%)	Male n (%)	Total n (%)	*P*-value for "Total"
20-30	1 (6.25)	2 (22.2)	3 (12.0)	0	0	0	1 (9.1)	0	1 (5.0)	*P*=0.218
	P=0.530						*P*=1.0			
31-40	2 (12.5)	1 (11.1)	3 (12.0)	2 (16.7)	1 (10)	3 (13.6)	2 (18.2)	1 (11.1)	3 (15.0)	*P*=0.957
	P=1.0			*P*=1.0			*P*=1.0			
41-50	1 (6.25)	1 (11.1)	2 (8.0)	1 (8.3)	2 (20)	3 (13.6)	1 (9.1)	0	1 (5.0)	*P*=0.606
	P=1.0			*P*=0.571			*P*=1.0			
51-60	2 (12.5)	0	2 (8.0)	1 (8.3)	1 (10)	2 (9.1)	1 (9.1)	2 (22.2)	3 (15.0)	*P*=0.724
	P=0.520			*P*=1.0			*P*=0.566			
61-70	4 (25.0)	3 (33.3)	7 (28.0)	7 (58.3)	4 (40)	11 (50)	5 (45.5)	4 (44.4)	9 (45.0)	*P*=0.270
	P=0.673			*P*=0.670			*P*=1.0			
71-80	5 (31.2)	1 (11.1)	6 (24.0)	0	1 (10)	1 (4.5)	1 (9.1)	1 (11.1)	2 (10.0)	*P*=0.129
	P=0.364			*P*=0.454			*P*=1.0			
≥ 80	1 (6.25)	1 (11.1)	2 (8.0)	1 (8.3)	1 (10)	2 (9.1)	0	1 (11.1)	1 (5.0)	*P*=0.873
	P=1.0			*P*=1.0			*P*=0.450			
Total	16 (100)	9 (100)	25 (100)	12 (100)	10 (100)	22 (100)	11 (100)	9 (100)	20 (100)	

P=0.634 for "Total" by age/severity

Discussion

The current study evaluated the prevalence and seriousness of KI in COVID-19 patients to ascertain how it affected clinical assumptions. A total of 190 COVID-19 patients were admitted to the central laboratory at AL-Saddar Teaching Hospital/Najaf, Iraq, between June 1 and August 20, 2020. The total prevalence rate of AKI was 35.3% out of 190 COVID-19 patients. About of 46.0% were hospitalized in the ICU, compared to 15.4% patients without AKI. In addition, 22.4% of COVID-19 patients needed dialysis, and 13.4% died. According to a comprehensive evaluation of 24 publications on 4963 COVID-19 patients, the AKI incidence rate was 4.5%. The outcomes of the present investigation were much higher than in this study. Conversely, the current incidence rate is somewhat like a US report, which detected 36.6% of patients with AKI.[11] Although most patients have mild to moderate symptoms, COVID-19 is consistently associated with morbidity and mortality from respiratory failure, acute respiratory distress syndrome, and sepsis.[11] Some studies reported the variation in the prevalence rate of kidney injury among COVID-19 patients ranged from 4.7% to 74.6%.[12,13] These differences across research findings can be due to various elements, the most crucial of which are the quality of healthcare system centers, the extent of challenges of the centers, the randomized controlled trials scheme with viral diagnostics, municipal methods, unobtainable therapeutic approaches, and limiting hospital stay practices.[14]

In COVID-19 patients under the current investigation, hypertension was considerably linked to the emergence of kidney injury, and this outcome is comparable to a Chinese investigation of 394 patients. The researchers noted that hypertension markedly contributed to AKI.[15] The severity of the injury could explain the link between hypertension and AKI. Similarly, in a Chinese cohort study of 1389 patients, a history of hypertension was more prevalent among several patients who suffered from severe kidney injury than among the COVID-19 patients who did not have kidney injury.[16,17]

This study also demonstrated that D-dimer and CRP serum levels were considerably greater in AKI patients than in those who did not have AKI. Other researchers noted that CRP levels higher than 10 mg/L were directly correlated with kidney injury. In another study,[18]) they examined 555 patients retrospectively and noticed that AKI patients had greater levels of D-dimer and CRP. In patients with kidney disease, a high D-dimer level may mirror the severity of the disease, minimize its eradication by the kidneys, and stimulate agglutination. Even so, decreasing kidney function has been linked to higher levels of other thrombolytic markers, such as soluble plasmin, vimentin, factor VIII levels, globulin, and fibrin complex.[11] The connection between CRP and AKI is challenging. Following some scientific evidence CRP stimulates the signaling pathway and down-regulates RANTES (regulated upon activation, normal T cell expressed and secreted [also known as CCL5]) expression. That also plays a crucial role in attracting inflammatory cells into sites of inflammation in human kidney distal tubular cells in a dose-responsive manner.[11]

This study also identified that the females were slightly more often exposed to AKI than the males. Additionally, there were no noticeable differences between the age groups. According to a descriptive cross-sectional study of 957 patients with COVID-19 at the Chest Disease Hospital in Kashmir,[18] in the age group >60 years, the severe cases of illness were 35.74%, and the incidence of death was 10.49%. The severe cases of illness were 32.39% in males, with a mortality rate of 11.27%. The severity of the illness was found to be 33.96% in females, and the occurrence of death was 12.58%.

The lack of adequate hospital facilities and difficulty accessing the hospital during the pandemic limited the collection of other lab examinations, such as urine test results and ventilator variables. These parameters could be crucial in the validation of the current findings. As a result, the present findings require additional support. Therefore, since this study was conducted in a local hospital in Najaf City during the COVID-19 disease outbreak, the results could not be generalized to all COVID-19-infected patients in Iraq.

Conclusion

The results indicated that AKI is prevalent in hospitalized COVID-19 patients and is attributed to in-hospital death rates. Health professionals in countries of particular concern, like Iraq, must raise their understanding of kidney damage in patients suffering from severe COVID-19 and concentrate primarily on protection and citizen education actions to employ COVID-19 precautionary actions. It is suggested that similar research with more patients and other areas of the country should be implemented.

Ethical Considerations

The study protocol was reviewed and approved by the Ethics Committees of the University of Kufa and the General Organization of Teaching Hospitals, Najaf City, Iraq.

Competing Interests

The authors declare that they have no competing interests.

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International Journal of Biomedicine 13(4) (2023) 301-305
http://dx.doi.org/10.21103/Article13(4)_OA11

ORIGINAL ARTICLE

COVID-19

INTERNATIONAL JOURNAL OF BIOMEDICINE

Some Aspects of Mast Cells Carboxypeptidase A3 Participation in the Pathogenesis of COVID-19

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Abstract

Background: This study aimed to determine the involvement of carboxypeptidase A3 (CPA3) in developing lung damage in patients with COVID-19.

Methods and Results: The study included samples of autopsy material from the lungs of patients who died as a result of severe COVID-19 (the main group [MG] and persons who died from external causes (the control group [CG]). Immunohistochemical staining for CPA3 was carried out. A quantitative study of CPA3-positive mast cells (MCs) and the degree of their degranulation was carried out using a ×40 objective lens with an analysis of ≥50 fields of view with further conversion to 1 mm². Significant representation of CPA3-positive MCs per 1 mm² of CPA3-positive MCs, CPA3-positive MCs with signs of degranulation (SD), and co-adjacent MCs was found in the MG compared to the CG (P=0.01 in all cases). In the main group, positive correlations were identified between the total number of CPA3-positive MCs, CPA3-positive MCs with SD and the blood hemoglobin level shortly before death (r=0.491 [P=0.008] and r=0.521 [P=0.004], respectively). Co-adjacent CPA3-positive MCs were negatively correlated with blood eosinophils at the beginning of hospitalization (r=-0.420 [P=0.023]). Also, the number of separately lying, CPA3-positive MCs negatively correlated with the blood monocyte shortly before death (r=-0.384 [P=0.044]). A positive correlation was established between the total number of CPA3-positive MCs, CPA3-positive MCs with SD, and adjacent CPA3-positive MCs with total blood protein in patients at the beginning of hospitalization (r=0.431 [P=0.020], r=0.449 [P=0.015] and r=0.456 [P=0.013], respectively). In addition, the study demonstrated a positive correlation between CPA3-positive MCs with SD and the total number of CPA3-positive MCs with blood aPTT levels (r=0.304 [P=0.045] and r=0.375 [P=0.045], respectively). A negative correlation was also found between the total number of CPA3-positive MCs and the blood INR level (r=-0.812 [P=0.050]). Finally, in patients at the beginning of hospitalization, a negative correlation was found between CPA3-positive MCs with SD, CPA3-positive MCs without SD, separately located CPA3-positive MCs, adjacent CPA3-positive MCs, and the total number of CPA3-positive MCs with blood amylase (r=-0.550 [P=0.002], r=-0.452 [P=0.045], r=-0.485 [P=0.030], r=-0.622 [P=0.008], and r=-0.590 [P=0.006], respectively).

Conclusion: Our study identifies the potential involvement of CPA3 in the pathogenesis of severe COVID-19. However, many aspects of its participation remain unclear and require further study. **(International Journal of Biomedicine. 2023;13(4):301-305.)**

Keywords: COVID-19 • SARS-CoV-2 • carboxypeptidase A3 • mast cells

For citation: Budnevsky AV, Avdeev SN, Ovsyannikov ES, Alekseeva NG, Shishkina VV, Savushkina IA, Perveeva IM, Feigelman SN, Kitoyan AG, Drobysheva VR. Some Aspects of Mast Cells Carboxypeptidase A3 Participation in the Pathogenesis of COVID-19. International Journal of Biomedicine. 2023;13(4):301-305. doi:10.21103/Article13(4)_OA11

Abbreviations

aPTT, activated partial thromboplastin time; **COVID-19**, coronavirus disease 2019; **CPA3**, carboxypeptidase A3; **CCL2**, chemokine (C-C motif) ligand 2; **G-CSF**, granulocyte colony-stimulating factor; **GBT**, general blood test; **INR**, international normalized ratio; **MCs**, mast cells; **SARS-CoV-2**, severe acute respiratory syndrome coronavirus 2; **SD**, signs of degranulation.

Introduction

COVID-19, an infectious disease caused by SARS-CoV-2, was first reported in 2019 and has already generated more than 700 million infections worldwide.[1] A hallmark of COVID-19 pathogenesis is a "cytokine storm" that causes elevated levels of pro-inflammatory cytokines and chemokines such as IL-6, TNF-α, CCL2, and G-CSF.[2] When a coronavirus enters the body, it primarily becomes attacked by innate immune cells—macrophages, lymphocytes, and mast cells (MCs)—strategically localized in the connective tissue of the membranes of the nasal cavity and lower respiratory tract.[3] MCs contain many biologically active substances packaged in cytoplasmic granules, such as a group of proteases, biogenic amines, and glycosaminoglycans. Many of them play a key role in the pathogenesis of COVID-19, exacerbating the inflammation process.[4] Carboxypeptidase A3 (CPA3) is also involved in the development of infectious and non-infectious diseases, and it is an important component of the MC's secret.[5,6] It has been found that CPA3 is involved in the pathogenesis of COVID-19. This may be explained by the abundant expression of CPA3 by MCs in various organs, including human lungs.[7,8]

This study aimed to determine the involvement of CPA3 in developing lung damage in patients with COVID-19.

Materials and Methods

The study included 30 patients (13(43%) men and 17(57%) women with an average age of 61.1±11.9 years) with a diagnosis of severe and extremely severe COVID-19, accompanied by bilateral, viral, community-acquired pneumonia, acute respiratory distress syndrome (ARDS) (diagnosed by the Berlin definition criteria),[9] who were treated at Voronezh Regional Clinical Hospital No. 1 in the COVID-19 departments from September 2021 to March 2022 and those who died as a result of COVID-19. Autopsy material from the lungs of patients in the main group (MG) was collected at the bases of pathology departments. The control group (CG) included 9 persons who died from external causes (4(44%) men and 5(56%) women; an average age of 60.9±10.1 years). Demographic indicators and the presence of comorbidities are presented in Table. 1.

The study did not include patients with chronic respiratory diseases (bronchial asthma, chronic obstructive pulmonary disease, chronic bronchitis, occupational lung diseases, other (except COVID-19) infectious respiratory diseases), cancer, hepatitis and cirrhosis, chronic kidney disease stage 3a and higher.

The collection of autopsy material from the lungs was carried out at the Voronezh Regional Bureau of Medical Examinations. The autopsy material was fixed in 10% neutral buffered formalin, a sample preparation procedure and embedded in paraffin, followed by the preparation of 5 μm thick sections for staining with H&E and Giemsa solution and ultrathin sections 2 μm thick for immunohistochemical analysis. Immunohistochemical staining was carried out according to the standard protocol[10] using polyclonal rabbit antibodies to CPA3 from Abcam (catalog number ab251685) at a dilution of 1:1000; after the application of secondary antibodies, sections were placed in a mounting medium. Microspecimens were analyzed using a Zeiss Axio Imager microscope A2 (Carl Zeiss, Germany) with a photo documentation system for images and a digital camera, Axiocam 506 color (Carl Zeiss, Germany). Images were processed in the ZEN 2.3 program. A quantitative study of CPA3-positive MCs and the degree of their degranulation was carried out using a ×40 objective lens with an analysis of ≥50 fields of view with further conversion to 1 mm². In patients of the MG, upon admission to the hospital and at least once over time, GBT and blood biochemistry were performed.

Table 1.

Patient demographic indicators and the presence of comorbidities.

Parameter	MG (n=30)	CG (n=9)
Sex, n (%) • male • female	 13 (43.3) 17 (56.7)	 4 (44.4) 5 (55.5)
Age, years	61.1 ± 11.9	60.9 ± 10.1
PCR SARS-CoV-2 «+», n (%)	30 (100)	0 (0)
Bilateral pneumonia, n (%)	30 (100)	0 (0)
ARDS, n (%)	30 (100)	0 (0)
Type 2 Diabetes, n (%)	1 (3.3)	0 (0)
Arterial hypertension, n (%)	25 (83.3)	8 (88.9)
Ischemic heart disease, n (%)	2 (6.7)	1 (11.1)
Ischemic stroke, n (%)	4 (13.3)	1 (11.1)
Chronic heart failure, n (%)	7 (23.3)	3 (33.2)
Obesity, n (%)	8 (26.7)	0 (0)
Chronic kidney disease, n (%)	2 (6.7)	1 (11.1)

Statistical analysis was performed using STATGRAPHICS Centurion XV software. The normality of data distribution was assessed using skewness, kurtosis, Kolmogorov–Smirnov, and Shapiro-Wilk tests. For descriptive analysis, results were presented as mean±standard deviation (SD), median (Me), lower quartile (Q1) and upper quartile (Q3). The Mann-Whitney U test was used to compare the differences between the two independent groups. Pearson's and Spearman's correlation coefficients were calculated to measure the strength and direction of the relationship between two variables. A probability value of $P<0.05$ was considered statistically significant.

Results and Discussion

The study determined the average number of CPA3-positive MCs per 1mm² in the MG and the CG. In addition, the number of CPA3-positive MCs with SD, CPA3-positive MCs without SD, MC fragments, and joint adherence of MCs were assessed (Table 2).

In the lung tissues of patients with COVID-19, it was found wide representation of CPA3-positive MCs with variable

degrees of degranulation activity or without it (Figure 1). Significant representation of CPA3-positive MCs per 1mm² of CPA3-positive MCs, CPA3-positive MCs with SD, and co-adjacent MCs was found in the MG compared to the CG (*P*=0.01, *P*=0.001, and *P*=0.0001, respectively).

Table 2.

CPA3-positive MCs in lung tissues of patients in MG and CG.

Parameter	Main group (n = 30)	Control group (n = 9)	*P*
CPA3-positive MCs without SD, per 1 mm²	4.35 (1.8;7.2)	3.48 (2.3;4.1)	0.796
CPA3-positive MCs with SD, per 1 mm²	6.49 (1.1;11.1)	2,07 (1.0;2.7)	0.001
MC fragments, per 1 mm²	1.52 (0.1;2.3)	1.27 (0.57;1.7)	0.779
Co-adjacent MCs, per 1 mm²	0.41 (0.1;0.5)	0.06 (0.0;0.1)	0.0001
Total number of MCs, per 1 mm²	10.84 (3.5;19.8)	5.56 (4.2;6.8)	0.01

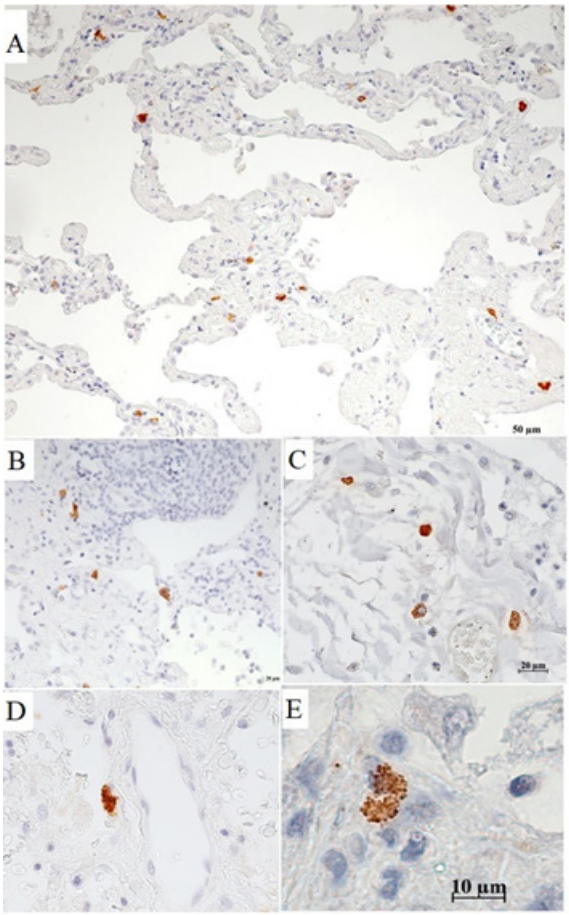

Fig. 1. *Histo-topographic features of CPA3-positive MCs in the lung tissues of patients without COVID-19 (A) and with COVID-19 (B-D). Immunohistochemical reaction: specific brown staining, nuclei stained blue with Mayer's hematoxylin.*
A – MCs with carboxypeptidase granules, with different levels of secretory activity (without degranulation and with signs of degranulation); B – interalveolar septa are deformed and thickened due to inflammatory cell infiltration, surrounded by CPA3-positive MCs with SD; C - area of developing pulmonary fibrosis, modified by CPAZ-positive MCs; D – perivascular localization of a CPAZ-positive MC; E – intercellular interaction of CPAZ-positive MC. Magnification: A – x200; B, C, D – x400, D – x1000.

Positive correlations were identified between the total number of CPA3-positive MCs, CPA3-positive MCs with SD and the blood hemoglobin level in GBT performed on the patient shortly before death (r=0.491 [*P*=0.008] and r=0.521 [*P*=0.004], respectively). CPA3-positive MCs in autopsy lung material also showed correlations with blood eosinophils. The average number of co-adjacent CPA3-positive MCs per 1mm² was negatively correlated with blood eosinophils at the beginning of hospitalization (r=-0.420 [*P*=0.023]). Also, the number of separately lying, CPA3-positive MCs negatively correlated with the blood monocyte shortly before death (r=-0.384 [*P*=0.044]). A positive correlation was established between the total number of CPA3-positive MCs, CPA3-positive MCs with SD, and adjacent CPA3-positive MCs with total blood protein in patients at the beginning of hospitalization (r=0.431 [*P*=0.020], r=0.449 [*P*=0.015], and r=0.456 [*P*=0.013], respectively). In addition, the study demonstrated a positive correlation between CPA3-positive MCs with SD and the total number of CPA3-positive MCs with blood aPTT levels (r=0.304 [*P*=0.045] and r=0.375 [*P*=0.045], respectively). A negative correlation was also found between the total number of CPA3-positive MCs and the blood INR level (r=-0.812 [*P*=0.050]). Finally, in patients at the beginning of hospitalization, a negative correlation was found between CPA3-positive MCs with SD, CPA3-positive MCs without SD, separately located CPA3-positive MCs, adjacent CPA3-positive MCs, and the total number of CPA3-positive MCs with blood amylase (r=-0.550 [*P*=0.002], r=-0.452 [*P*=0.045], r=-0.485 [*P*=0.030], r=-0.622 [*P*=0.008], and r=-0.590 [*P*=0.006], respectively).

The results of our previously published study revealed that in patients with COVID-19, the average number of tryptase-positive MCs without SD and the total number of CPA3-positive MCs was statistically significantly higher, and tryptase fragments and CPA3-positive MCs were lower than in the CG. Negative correlations were established between the number of tryptase-positive MCs and the content of erythrocytes in GBT. A negative correlation was found between the number of non-degranulating tryptase-positive MCs and the hemoglobin content. Positive correlations were found between tryptase-positive MCs and the content of leukocytes in GBT, and negative correlations between the number of CPA3-positive MCs and the platelet content (*P*=0.0436 and *P*=0.0334, respectively). A direct correlation was established between the number of co-adjacent and fragments of tryptase-positive MCs with the erythrocyte sedimentation rate. A negative correlation was found between the number of CPA3-positive MCs without SD and the level of blood C-reactive protein (*P*=0.0278). In patients with COVID-19, reduced degranulation activity of tryptase-positive MCs was found, along with an increased representation of CPA3-positive MCs.[11] It was also revealed that the total number of CPA3-positive MCs per 1mm² in autopsy lung material obtained from patients with COVID-19 was significantly higher than in the CG, which may indicate the involvement of CPA3 in the pathogenesis of patients with COVID-19.

In the current study, negative correlations were observed between separately lying, CPA3-positive MCs and the content

of blood monocytes. Evidence suggests that in SARS-CoV-2 infection, monocytes, macrophages, and MCs can produce large amounts of multiple types of proinflammatory cytokines and chemokines, causing a cytokine storm with local tissue inflammation and a dangerous systemic inflammatory response. Low expression of ACE2 by monocytes/macrophages in COVID-19 patients may also contribute to the development of pathological reactions due to the proinflammatory properties of angiotensin II and dysfunction of the renin-angiotensin system. Both local tissue inflammation and cytokine storm play a fundamental role in the development of complications associated with COVID-19, such as ARDS, which is the leading cause of death in patients infected with SARS-CoV-2.[12] In addition, chymase and CPA3 can also interact in the enzymatic cleavage of angiotensin II, in which each enzyme has its catalytic activity toward specific substrate proteins.[13] Co-adjacent CPA3-positive MCs in autopsy lung material showed positive correlations with the content of eosinophils in SGBT. Subsets of MCs containing CPA3 have previously been reported to be involved in major airway diseases, such as asthma, COPD, and pulmonary fibrosis.[8,14,15]

The identified positive correlations between the total number of CPA3-positive MCs and CPA3-positive MCs with SD with the blood hemoglobin level are consistent with existing data that SARS-CoV-2 infection significantly affects the structural membrane homeostasis of erythrocytes at the levels of protein and lipids. In the red blood cells of COVID-19 patients, the levels of glycolytic intermediates were increased, accompanied by oxidation and fragmentation of membrane proteins. In patients with COVID-19, red blood cells may be unable to respond to changes in hemoglobin oxygen saturation as they move from the lungs into the bloodstream and may have a reduced ability to transport and deliver oxygen.[16]

Noteworthy is the established positive correlation between the number of CPA3-positive MCs with SD and the total number of CPA3-positive MCs with the aPTT level and the negative correlation between the total number of CPA3-positive MCs and the blood INR level. The transition from mild to severe disease in patients with COVID-19 may be caused by a cytokine storm and increased hypercoagulability with a significant risk of thromboembolic complications.[17] COVID-19 causes endothelial damage, coagulation activation, and intravascular fibrin deposition. Patients experienced thrombocytopenia, elevated D-dimer levels, and prolonged aPTT, suggesting that death in patients with COVID-19 may be related to disseminated intravascular coagulation.[18]

The data obtained are in line with data confirming that CPA3 has potential significance for pulmonary fibrosis, COPD, as it regulates the contraction of smooth muscles, regulates blood vessel tone and vascular blood flow through proteolytic modification, for example, angiotensin I, apolipoprotein B and neurotensin. Even with the proposed role of CPA3 in homeostasis, activated CPA3 mRNA may also have pro-inflammatory effects and is essential for the biogenesis of extracellular matrix components.[19]

Finally, the discovered negative correlation between CPA3-positive MCs with SD, CPA3-positive MCs without SD, separately located CPA3-positive MCs, adjacent CPA3-positive MCs, and the total number of CPA3-positive MCs with blood amylase is confirmed by the data that SARS-CoV-2 infection contributes to pancreatic damage. The virus infects the endocrine part of the pancreas and, to a lesser extent, the exocrine part. It has been shown that there is a bidirectional relationship between COVID-19 and diabetes; patients with COVID-19 and concomitant diabetes mellitus had a severe and extremely severe course of the disease and increased mortality; in other patients with COVID-19, without concomitant diabetes mellitus, the earlier development of COVID-19 was observed.[20]

In conclusion, our study identifies significant correlations between CPA3-positive MCs and the level of monocytes, eosinophils, hemoglobin, amylase, total protein, INR, and aPTT. A statistically significant increased total number of CPA3-positive MCs, CPA3-positive MCs with SD, and co-adjacent MCs was found in the MG compared to the CG. The potential involvement of CPA3 in the pathogenesis of COVID-19 was considered, namely in changes in hematological parameters and blood coagulation parameters, inflammation, pulmonary fibrosis, and organ failure observed in COVID-19.

Competing Interests

The authors declare that they have no competing interests.

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International Journal of Biomedicine 13(4) (2023) 306-311
http://dx.doi.org/10.21103/Article13(4)_OA12

ORIGINAL ARTICLE

Dentistry

INTERNATIONAL JOURNAL OF BIOMEDICINE

Oral Changes in Chronic Renal Failure Patients in One of the Regional Hospitals in Kosovo

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Abstract

Chronic kidney disease (CKD) patients have a higher tendency to have oral diseases such as periodontitis, saliva flow changes, bleeding gums, dry mouth, and bad breath. Malnutrition, oxidative stress, and vitamin deficiency, such as complex vitamin B and vitamin C, are the main factors that may cause oral changes. This study aimed to evaluate the subjective oral health of a sample of 90 patients with chronic renal failure (CRF) in dialysis and determine the relationship between renal failure and oral changes. As a result, the most frequent answer was dry mouth after dialysis at around 73.3% of the patients, whereas 41.1% had tooth decay, 52% had bad breath, 35% had gingival bleeding and 22.2% have noticed tooth coloring. The comparison between male and female patients found significant differences in uremic fetor: male patients had an average score of 1.58, while female patients had 1.36 (P=0.0371). Another significant difference was found for tooth discoloration: the average score for males was 1.67 and for females, 1.90 (P=0.0082). Patients in urban areas had an average score for caries after dialysis of 1.40, while those in rural areas had 1.68 (P=0.01). Patients with CRF should get multidisciplinary treatment. These patients require special consideration not only about dental treatment but also because of the side effects of the treatments they receive. A detailed evaluation and provision of good oral care after diagnosis of end-stage renal disease is more than necessary.**(International Journal of Biomedicine. 2023;13(4):306-311.)**

Keywords: chronic renal failure • uremic fetor • dry mouth

For citation: Muhaxheri G, Kelmendi J, Hysenaj Cakolli V, Kolgeci D. Oral Changes in Chronic Renal Failure Patients in One of the Regional Hospitals in Kosovo. International Journal of Biomedicine. 2023;13(4):306-311. doi:10.21103/Article13(4)_OA12

Abbreviations

CKD, chronic kidney disease; **CRF**, chronic renal failure

Introduction

Numerous systemic diseases can affect patients' oral health. The prevalence of CKD worldwide is significantly increasing; therefore, oral health issues in these patients should be considered, since there are side effects of the drug intake and dental treatment, and so should the relationship between malnutrition, vitamin insufficiency, oxidative stress, and oral changes that may be caused.[1,2] Mostly, some of these factors are related to the stage of the disease; furthermore, at the higher stage of the disease, oral health issues are more present. In recent studies, almost 90% of patients with CKD have oral health issues.[3] Reducing erythropoietin causes anemia, which causes atrophic tongue, petechiae, ecchymoses, and angular cheilitis.[4] The most common symptom in oral health in these patients is dry mouth, which can be caused by limited fluid intake (necessary to adjust the capacity of reduced renal failure), side effects of drug therapy, and decreased flow of saliva.[5] Patients also suffer from ammonia breath and a sensation of metallic taste in the mouth. This occurs because of high urea in the saliva that turns into ammonia, or another cause may be acidic saliva.[5]

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Furthermore, gingival inflammation has been reported to be caused by acidic saliva combined with poor oral hygiene.[6] Uremic stomatitis is a condition that appears in patients with high uremic blood values of 300 mg/ml, and it has 2 forms: erythematosus form, which is characterized by burning mucositis, and pseudomembranous form, which is ulcerative and characterized by burning ulcers.[7]

Uremic frostbite occurs from crystal uremic formation in epithelial surfaces.[8] Candidiasis is also present in some patients because of decreased immunity, especially in patients with kidney transplants.[9] Enamel hypoplasia in the form of white and brown discoloration usually is seen in early renal disease; therefore, patients may notice spotting and brown teeth due to uremia and iron supplements.[10]

Renal osteodystrophy results from disorders in the metabolism of calcium, phosphorus, or vitamin D and increased parathyroid activity.[11] Intestinal absorption of calcium decreases in chronic renal failure (CRF) because the kidneys cannot convert vitamin D into its active form (1,25 dihydroxycholecalciferol). There is a corresponding phosphate retention, which ultimately leads to decreased serum calcium levels. This situation is accompanied by compensatory hyperactivity of the parathyroid gland, leading to increased secretion of urinary phosphates, decreased urinary calcium excretion, and increased calcium release from the bones.[10,12]

Metabolic renal osteodystrophy and compensatory hyperparathyroidism manifest in ways that include reduced trabeculae, demineralization, and appearance.[10] Other significant changes are reduction of cortical bone thickness, loss of lamina dura, lesions of radiolucent giant cells, expansion of the underlying skeleton, and metastatic soft tissue calcification.[13] Patients have an increased risk of jaw fracture due to trauma or oral intervention.[14] Other symptoms include healing disorders after tooth extraction, mobility of the tooth, gnathic disorders, hypoplasia, and denticles.[15] Radiographically, it is observed that the resorption of lamina dura leads to osteodystrophy.[16] Close cooperation between the dentist and nephrologists is required to treat patients.[17] Early assessment of the oral health status of patients with kidney disease is essential to eliminate potential foci of infection from the oral cavity. Before any surgery, patients must undergo a detailed oral assessment, and any necessary dental treatment should be planned and carried out carefully.

Materials and Methods

Data was retrieved from patients in the Regional Hospital of Prizren, a city in Kosovo. In the research, a total of 90 patients participated. Among them, 48 were male (53.3%), while 42 were female (46.7%).

We used the following patient inclusion criteria: the diagnosis of verified CRF with anamnesis, physical examination, and completion of the dialysis criterion. Data related to patients' disease was gathered with an anonymous survey with 26 informative questions. We divided these questions into 3 groups: one group related to general information about patients, the second group related to information about CRF, and the third set related to oral health and possible oral manifestations.

Patients were informed about the purpose of the study questionnaire, participated voluntarily in the study, and gave informed verbal consent. An anonymous personal interview with patients was conducted by the clinician. Only patients who were able to follow the questions and offer answers were interviewed. Patients were interviewed only once during the study. There were no exceptions in the selection of patients regarding disease stage, age, and type of treatment at the time of the interview.

Statistical analysis was performed using statistical software package SPSS version 23.0 (SPSS Inc, Armonk, NY: IBM Corp). The normality of distribution of continuous variables was tested by the Kolmogorov-Smirnov test with the Lilliefors correction and Shapiro-Wilk test. For the descriptive analysis, results are presented as mean (M) ± standard deviation (SD)/ standard error of the mean (SEM). For data with normal distribution, inter-group comparisons were performed using Student's t-test. Differences of continuous variables departing from the normal distribution, even after transformation, were tested by the Mann-Whitney U-test. Pearson's Correlation Coefficient (r) was used to determine the strength of the relationship between the two continuous variables. A probability value of $P<0.05$ was considered statistically significant.

Results

In the research, a total of CRF 90 patients (48 male and 42 female) participated. Of them, 36(40%) had a low secondary education, 47(52.2%) had a higher secondary education, and 7(7.8%) had a university education. Among them, 7(7.8%) were unmarried and 83(92.2%) were married. Regarding the place of residence, 30(33.3%) were from the city, and 60(66.7%) were from the rural areas (Table 1).

Table 1.

Baseline characteristics of study population.

Educational level	N	%
Low secondary education	36	40.0
Higher secondary education	47	52.2
University education	7	7.8
Marital status	N	%
Single	7	7.8
Married	83	92.2
Place of residence	N	%
Urban	30	33.3
Rural	60	66.7
Gender	N	%
Male	48	53.3
Female	42	46.7

Regarding uremic fetor, 47(52.2%) responded with "Yes" and 43(47.8%) responded with "No"; 23(25.6%) patients had

a metallic taste, while 66(73.3%) did not; and 1(1.1%) did not respond. Thirty-two (35.6%) of the respondents expressed having gingival bleeding, while 58(64.4%) did not. Regarding the frequency of toothbrush replacement, 24(26.7%) stated that they replace it every 3 months, 27(30%) every 6 months, 35(38.9%) every year; 3(3.3%) do not have toothbrushes, and 1(1.1%) did not provide an answer. Twenty (22.2%) patients color their teeth, while 70(77.8%) do not. Many respondents (42.2%) feel thirsty during the day, while 36(40%) feel thirsty after dialysis, and fewer (16.7%) experience thirst at night. Sixty-six (73.3%) patients stated they had a dry mouth, while 24(26.7%) did not. After dialysis, 37(41.1%) have tooth decay, while 53(58.9%) do not (Table 2).

Table 2.

Oral subjective characteristics and changes in patients with CRF.

Uremic fetor	N	%
Yes	47	52.2
No	43	47.8
Metallic taste	N	%
Yes	23	25.6
No	66	73.3
N/A	1	1.1
Gingival bleeding	N	%
Yes	32	35.6
No	58	64.4
Frequency of toothbrush replacement	N	%
Every 3 months	24	26.7
Every 6 months	27	30.0
Every year	35	38.9
No toothbrush	3	3.3
N/A	1	1.1
Bad breath	N	%
Alcohol	1	1.1
Tobacco	16	17.8
Tobacco, Alcohol	1	1.1
No	72	80.0
Teeth staining	N	%
Yes	20	22.2
No	70	77.8
When you feel most thirsty?	N	%
During dialysis	1	1.1
During the day	38	42.2
During the night	15	16.7
After dialysis	36	40.0
How often do you brush your teeth?	N	%
1 time a day	46	51.1
2 times a day	39	43.3
3 times a day	1	1.1
Never	4	4.4
Dry mouth	N	%
Yes	66	73.3
No	24	26.7
Tooth with caries after dialysis	N	%
Yes	37	41.1
No	53	58.9

Table 3.

The comparison between male and female patients with CRF in terms of oral changes.

Parameter	Gender	N	Mean	SD	SEM
Quality of life with dialysis	Male	47	1.11	0.312	0.045
	Female	42	1.14	0.354	0.055
Last dental visit	Male	48	2.77	1.259	0.182
	Female	42	2.81	1.254	0.194
Uremic fetor*	Male	48	1.58	0.498	0.072
	Female	42	1.36	0.485	0.075
Metallic taste	Male	47	1.79	0.414	0.060
	Female	42	1.69	0.468	0.072
Gingival bleeding	Male	48	1.69	0.468	0.068
	Female	42	1.60	0.497	0.077
Brush replacement frequency	Male	48	3.23	0.951	0.137
	Female	42	3.10	0.850	0.131
Bad breath***	Male	48	3.29	0.988	0.143
	Female	42	3.95	0.309	0.048
Teeth staining**	Male	48	1.67	0.476	0.069
	Female	42	1.90	0.297	0.046
When do you feel most thirsty?	Male	48	2.83	0.930	0.134
	Female	42	3.10	0.932	0.144
How often do you brush your teeth?	Male	48	1.67	0.859	0.124
	Female	42	1.50	0.552	0.085
Dry mouth	Male	48	1.29	0.459	0.066
	Female	42	1.24	0.431	0.067
Tooth with caries after dialysis	Male	48	1.60	0.494	0.071
	Female	42	1.57	0.501	0.077

** - P=0.0371, ** - P=0.0082, *** - P=0.000.*

In the following results, we analyzed the comparison between male and female patients in terms of Quality of Life on Dialysis, Last Dental Visit, Uremic Fetor, Metallic Taste, Gingival Bleeding, Frequency of Brushing, Bad Breath, Tooth Discoloration, Thirst Sensation, Frequency of Tooth Cleaning, Dry Mouth, and Tooth Decay after Dialysis (Table 3). Based on the descriptive results, we can see that in terms of Quality of Life on Dialysis, male patients had an average score of 1.11, while female patients had 1.14. Regarding the Last Dental Visit, male patients had a slightly lower average score of 2.77 than females (2.81). In terms of Fetor, males had an average score of 1.58, while females had 1.36. For Metallic Taste, males had an average score of 1.79, females 1.69. There were also slight differences in Gingival Bleeding, where males had an average score of 1.69 whereas females had an average score of 1.60. Similarly, in the Frequency of Brushing, males had an average score of 3.23, while females had 3.10. In terms of Tooth Discoloration, males had an average score of 1.67,

while females had 1.90. As for Thirst Sensation, males had an average score of 2.83, while females had 3.10. The average frequency of Tooth Cleaning for males was 1.67, whereas for females, it was 1.50.

Significant differences have been found in Uremic Fetor. Male patients had an average score of 1.58, while female patients had 1.36 (P=0.0371). Significant differences were also found in Bad Breath. Male patients had an average score of 3.29, while female patients had 3.95 (P=0.000). We found another significant difference for Tooth Discoloration. The average score for males was 1.67 and for females, 1.90 (P=0.0082).

Below is presented the comparison between patients from urban and rural areas (Table 4), where, based on the descriptive results, we have a more pronounced difference in teeth with caries after dialysis. Patients in urban areas had an average score of 1.40, while those in rural areas had 1.68 *(P*=0.01). In other cases, there are no significant differences between patients from urban and rural areas.

Table 4.

The comparison between CRF patients from urban and rural areas in terms of oral change.

Parameter	Residence	N	Mean	SD	SEM
Quality of life with dialysis	Urban	30	1.07	0.254	0.046
	Rural	59	1.15	0.363	0.047
The last visit to the dentist	Urban	30	2.40	1.303	0.238
	Rural	60	2.98	1.186	0.153
Uremic fetor	Urban	30	1.40	0.498	0.091
	Rural	60	1.52	0.504	0.065
Metallic taste	Urban	30	1.70	0.466	0.085
	Rural	59	1.76	0.429	0.056
Gingival bleeding	Urban	30	1.63	0.490	0.089
	Rural	60	1.65	0.481	0.062
Brush replacement frequency	Urban	30	3.03	0.850	0.155
	Rural	60	3.23	0.927	0.120
Bad breath	Urban	30	3.60	0.814	0.149
	Rural	60	3.60	0.827	0.107
Teeth staining	Urban	30	1.67	0.479	0.088
	Rural	60	1.83	0.376	0.049
When you feel most thirsty	Urban	30	2.87	0.973	0.178
	Rural	60	3.00	0.921	0.119
How often do you brush your teeth?	Urban	30	1.70	0.651	0.119
	Rural	60	1.53	0.769	0.099
Dry mouth	Urban	30	1.20	0.407	0.074
	Rural	60	1.30	0.462	0.060
Tooth with caries after dialysis*	Urban	30	1.40	0.498	0.091
	Rural	60	1.68	0.469	0.061

*- *P=0.01*

In our study, we found significant differences only in the case of teeth with caries after dialysis between patients from urban and rural areas. Indeed, there was a correlation between patients with gingival bleeding and teeth with cavities after dialysis (r=0.323, 2-tailed P=0.002). This indicates that gingival bleeding increases the likelihood of having teeth with cavities after dialysis.

Discussion

The present study found that the most common symptom in patients with CRF is dry mouth followed by bad breath and tooth decay. Previous studies show that patients with CRK have a higher incidence of oral conditions.[18-20] Dry mouth in patients who are attending hemodialysis can be caused by uremia and dehydration because of the restriction of fluid intake.[21,22] Meanwhile, uremia is one of the dominant factors that can lead to decreased lymphocyte response, damage of granulocytes, and immunity suppression.[23,24]

Decreased salivary flow has an impact on dry mouth and halitosis. Also, it damages the patients' quality of life since they have speaking, chewing, and swallowing problems.[25-28] Bad breath is one of the most common subjective and objective symptoms in patients with CRF. The measurements that are made with organoleptic tests and gas halitosis were observed. Furthermore, hydrogen sulfide tends to be one of the most significant factors that cause halitosis that comes from tongue coating.[29]

Kao et al.,[21] in their study, concluded that there is significantly poorer salivary function in patients with end-stage renal disease, which is compatible with our results. Furthermore, in our study, tooth decay, according to patients, is increased. Contrary to our findings, Chao et al. found that tooth decay doesn't seem to undergo any changes; even though there was high colonization of *Streptococcus mutans* it was not an indication of increased caries.[30] In most studies, salivary urea is observed to be one of the most important factors that increases the pH of the saliva and protects the tooth from demineralization.[31,32]

The present study shows the possible oral symptoms in patients with renal failure that attend hemodialysis. According to the study, oral manifestations are frequent, and among the affecting factors are age, disorders from kidney disease and/or accompanying diseases, side effects of the treatments they receive, and poor oral hygiene. People over 60 are more affected by the side effects of drugs used in therapy and the changes in the body that accompany this age. This study has limitations since we did not examine the patients, and further research should be done.

Conclusion

Patients undergoing dialysis treatment show marked oral changes and changes in the flow of saliva; therefore, it is necessary to offer treatment and supervision by the dental staff. Untreated dental infections in patients with renal failure may contribute significantly to morbidity, as they have a compressed immune system. The awareness of patients under

dialysis treatment should be increased. Dentists should be informed about possible oral manifestations and prevention.

Competing Interests

The authors declare that they have no competing interests.

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International Journal of Biomedicine 13(4) (2023) 312-316
http://dx.doi.org/10.21103/Article13(4)_OA13

ORIGINAL ARTICLE

Dentistry

INTERNATIONAL JOURNAL OF BIOMEDICINE

Oral Lichen Planus and Thyroid Disease: A Case-Control Study

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Abstract

***Background*:** Oral lichen planus (OLP) is a chronic inflammatory condition that primarily affects the oral mucosa. While the exact cause of OLP remains unclear, recently, there has been particular interest in researching the potential link between OLP and thyroid gland disorders.

***Methods and Results*:** A total of 100 subjects participated in our research. Among them, 50 were patients (80% women and 20% men) diagnosed with OLP (the main group [MG]), and 50 subjects (60% women and 40% men) with various other oral mucosa concerns (aphthous stomatitis, burning mouth syndrome, herpetic stomatitis, and geographic tongue) comprised the comparison group [CG]. All participants underwent a comprehensive assessment of thyroid function, which included the measurement of free thyroxine (FT4), free triiodothyronine (FT3), thyroid-stimulating hormone (TSH), thyroid peroxidase antibodies (TPO-Ab), and an ultrasound examination of the thyroid gland performed by an endocrinologist.

Our results reveal a noteworthy gender-based difference in the occurrence of mucosal disorders, indicating a predominance of females. MG had a significantly higher prevalence of hypothyroidism, with 46% of patients affected. In contrast, in CG, the prevalence of hypothyroidism was lower, at 16%, and this difference was statistically significant (P=0.0012). In MG, 50% of cases were found to be in a euthyroid state. In contrast, in CG, a larger proportion (80%) of patients were in a euthyroid state, and this difference was statistically significant (P=0.0017).

Conclusion: Our study has identified a significant and positive association between hypothyroidism and OLP. We recommend that individuals diagnosed with OLP, especially women, consider undergoing routine screening for thyroid disease as part of their healthcare regimen.**(International Journal of Biomedicine. 2023;13(4):312-316.)**

Keywords: oral lichen planus • mucosa • hypothyroidism

For citation: Krasniqi MS, Dalipi ZS, Zhjeqi V. Oral Lichen Planus and Thyroid Disease: A Case-Control Study. International Journal of Biomedicine. 2023;13(4):312-316. doi:10.21103/Article13(4)_OA13

Abbreviations

FT3, free T3; **FT4**, free T4; **OLP**, oral lichen planus; **TSH**, thyroid-stimulating hormone; **T4**, thyroxine; **T3**, triiodothyronine; **TSHR**, thyroid-stimulating hormone receptor.

Introduction

Oral lichen planus (OLP) is a persistent, non-malignant condition affecting oral mucosa.[1] This condition is relatively rare, accounting for less than 1% of oral cavity disorders.[2] While its exact cause remains unknown, there is evidence suggesting a potential genetic predisposition, as well as associations with certain medications, including NSAIDs, oral hypoglycemics, antimalarials, and psychotropic drugs. The use of tobacco has also been considered a potential contributing factor.[3,4] OLP predominantly affects females. Additionally, numerous studies have explored the possible link between thyroid disorders and the development of lichenoid changes in the oral mucosa, although the nature of this relationship remains unclear.[5-7]

Thyroid-stimulating hormone (TSH) is a hormone produced by the pituitary gland. It stimulates the thyroid gland to produce thyroxine (T4) and triiodothyronine (T3) through a negative feedback mechanism involving free T3 (FT3) and T4 (FT4).[6,8] The thyroid-stimulating hormone receptor (TSHR) presented in thyroid follicular cells is a key regulator of thyroid hormone synthesis and secretion.[9] Thyroid hormones are crucial for governing the body's growth, development, and metabolic processes. Thyroid disorders can disrupt the body's homeostasis and impact tissue healing capabilities.[10,11] Active TSHR has been identified in various tissues, such as osteoblasts, osteoclasts, bone marrow cells, cardiomyocytes, adipocytes, fibroblasts, and skin keratinocytes. However, the available information on thyroid protein expression in the oral mucosa is limited.[12,13]

Materials and Methods

A total of 100 subjects participated in our research. Among them, 50 were patients diagnosed with OLP (the main group [MG]), and they were assessed at the University Dental Clinical Center of Kosovo, having been referred by primary or secondary medical centers in Kosovo. To establish a basis for comparison, we also evaluated a comparison group [CG] of 50 subjects who presented with various other oral mucosa concerns, including conditions like aphthous stomatitis, burning mouth syndrome, herpetic stomatitis, and geographic tongue.

Inclusion Criteria: Participants in the study were required to be at least 18 years of age and should not have any pre-existing chronic illnesses. They were examined after providing written informed consent, and the examination findings were duly recorded.

Exclusion Criteria: Individuals with chronic coexisting medical conditions or autoimmune disorders, and pregnant women were not considered for inclusion in this research.

The diagnosis of OLP was based on both clinical and histological criteria. The clinical criteria involved the identification of specific features, including bilateral and symmetrical lesions. These lesions could manifest as net-like patterns with gray-white lines, in the form of white plates, or exhibit characteristics such as atrophy, blistering, or erosions.

Histological criteria were equally important and encompassed the presence of a well-defined band-like area of cellular infiltration restricted to the superficial layer of the connective tissue. Additionally, liquefaction degeneration in the basal cell layer was considered a significant histological marker. Importantly, the absence of epithelial dysplasia was also a crucial factor in diagnosing OLP.

In cases where the disease did not display typical clinical manifestations, tissue samples were sent for histopathological examination. All collected samples were fixed in a 10% formalin solution.

The WHO criteria were employed as the standard for clinical and histopathological assessments in diagnosing OLP.

Clinical Criteria

Presence of bilateral and symmetrical lesions.

Clinical manifestations may include reticular, erosive, atrophic, bullous, and plaque-type lesions.

Histological Criteria

Identification of a band-like area of lymphocytic infiltration within the superficial connective tissue.

Observance of signs of degeneration in the basal cell layer.

Absence of epithelial dysplasia.

Thyroid Gland Examination

All participants underwent a comprehensive assessment of thyroid function, which included the measurement of FT4, FT3, TSH, thyroid peroxidase antibodies (TPO-Ab), and an ultrasound examination of the thyroid gland performed by an endocrinologist.

Statistical analysis was performed using statistical software package SPSS version 21.0 (SPSS Inc, Armonk, NY: IBM Corp). Baseline characteristics were summarized as frequencies and percentages. Group comparisons were performed using chi-square tests or, alternatively, Fisher's exact test when expected cell counts were less than 5. A probability value of $P<0.05$ was considered statistically significant.

Results

Our results reveal a noteworthy gender-based difference in the occurrence of lichenoid changes, indicating a predominance of females (Table 1). This gender-based distinction was also observed in CG, where a significant difference was noted (Table 2). Table 3 summarizes the study's findings, indicating that 80% of cases exhibited manifestations of reticular lichen, primarily localized in the buccal mucosa, gingiva, tongue, and lip. Additionally, 74% of patients reported subjective symptoms, including burning sensations and oral mucosal discomfort. The thyroid gland function analysis in the study groups is presented in Table 4.

Table 1.

Distribution of the main group by age and gender.

Age	Gender*				Total	
	Female		Male			
	n	%	n	%	n	%
25-39	10	22.7	-	0.0	10	20.0
40-49	16	36.4	2	33.3	18	36.0
50-65	18	40.9	4	66.7	22	44.0
Total	44	100.0	6	100.0	50	100.0

**- P<0.0001 for Female versus Male.*

Prevalence of Hypothyroidism

MG had a significantly higher prevalence of hypothyroidism, with 46% of patients affected. In contrast, in CG, the prevalence of hypothyroidism was lower, at 16%, and this difference was statistically significant.

Table 2.

Distribution of the comparison group according to age and gender.

Age	Gender*				Total	
	Female		Male			
	n	%	n	%	n	%
25-39	7	23.3	10	50.0	17	34.0
40-49	11	36.7	6	30.0	17	34.0
50-65	12	40.0	4	20.0	16	32.0
Total	30	100.0	20	100.0	50	100.0

**- P=0.0466 for Female versus Male.*

Table 3.

Characteristics of OLP lesions in the main group.

Variable		n	%	Statistic
Clinical type	Reticular	40	80.0	$\chi2$=49.12 DF=2, *P*<0.0001
	Ulcerative & Erosive	6	12.0	
	Pllaque like	4	8.0	
Location	Buccal zone	46	92.0	$\chi2$=82.80 DF=3, *P*<0.0001
	Gingiva	6	12.0	
	Lip	4	8.0	
	Tongue	5	10.0	
Symptoms	Yes	37	74.0	$\chi2$=11.52 DF=1, *P*=0.0007
	No	13	26.0	
Gender	Female	44	88.0	$\chi2$=28.88 DF=1, *P*<0.0001
	Male	6	12.0	

Table 4.

The thyroid gland function analysis in the study groups.

Morbidity	MG		CG		Statistic
	n	%	n	%	
Hypothyroidism	23	46.0	8	16.0	$\chi2$=10.519 *P*=0.0012
Hashimoto Thyroiditis	2	4.0	2	4.0	Fisher's Exact Test, *P*=0.140
Euthyroid State	25	50.0	40	80.0	$\chi2$=9.89 *P*=0.0017
Total	50	100.0	50	100.0	

Hashimoto Thyroiditis

A small proportion (4%) of patients in MG had Hashimoto thyroiditis. This prevalence did not differ significantly from CG, where 4% of patients had Hashimoto thyroiditis.

Euthyroid State

In MG, 50% of cases were found to be in a euthyroid state. In contrast, in CG, a larger proportion (80%) of patients were in a euthyroid state, and this difference was statistically significant.

These findings highlight a higher prevalence of hypothyroidism in OLP patients than in CG, as well as differences in the euthyroid state between the two groups.

Discussion

OLP is a chronic inflammatory condition that primarily affects the oral mucosa, although, in some instances, it can also manifest as red papules on the skin.[14] Clinically, OLP is categorized into six forms: reticular, papular, plaque, erosive, atrophic, and bullous, with the reticular form being commonly observed within the oral cavity.[15]

While the exact cause of OLP remains unclear, it is known to be associated with several systemic disorders. Recently, there has been particular interest in researching the potential link between OLP and thyroid gland disorders.

Our study observed a higher prevalence of lichenoid lesions among female subjects, with 44 (88%) females affected compared to 6 (12%) males. These findings suggest a greater predilection for OLP among females with lichenoid lesions.

The findings of our study are consistent with previous research conducted by Tang et al.[16] and Xue et al.,[17] which also observed a higher prevalence of lichenoid lesions among female subjects.

Our study showed that the most common type of OLP was the reticular form, accounting for 80% of cases. This was followed by the erosive ulcerative form, present in 12% of cases, and the plaque form, observed in 8% of cases. These results align with a study by Baharvand et al.,[18] which also found a predominance of the reticular type of lichen.

Regarding the distribution of OLP lesions, our study showed that 92% of patients exhibited buccal mucosal involvement, followed by gingiva (12%), tongue (10%), and lip (8%). These findings align with the research conducted by Maddheshiya et al.,[19] which reported similar distribution patterns.

Additionally, our study revealed that 74% of patients with lichenoid lesions reported experiencing subjective symptoms, such as burning, discomfort, and sensation.

In our study, we observed a notable prevalence of hypothyroidism, characterized by low values of FT3 and FT4, in 23 out of 50 patients diagnosed with OLP, which accounts for 46% of this group. Additionally, two out of 50 OLP patients were found to have elevated TPO-Ab values, while the remaining 25 were in a euthyroid state. Importantly, when comparing these results to CG, we discovered that the prevalence of thyroid disorders, particularly hypothyroidism,

was significantly higher in patients with OLP, demonstrating a statistically significant difference (46% versus 16%).

Our findings align with the research conducted by Zhou et al.,[20] Robledo-Sierra et al.,[21] and Garcia-Pola et al.,[22] who also reported a strong correlation between OLP and hypothyroidism.

While our study did not identify a significant difference in the prevalence of Hashimoto's disease between the two groups, it is noteworthy that Alikhani et al.[23] reported that the severity of clinical expression of OLP lesions was directly linked to the levels of IL-8 in the serum, as well as the levels of TPO-Ab. This suggests that the severity of OLP symptoms in patients may be influenced by certain immune and inflammatory markers in the bloodstream, which could vary independently of Hashimoto's disease status.

We observed that 50% of MG patients were in a euthyroid state. In contrast, CG exhibited a higher proportion of patients (80%) in a euthyroid state. This difference suggests that thyroid function may impact the development or clinical expression of OLP. However, further research is needed to understand the relationship between thyroid function and OLP severity fully.

Conclusion

The relationship between OLP and thyroid diseases warrants further investigation and clarification. Our study has identified a significant and positive correlation between hypothyroidism and OLP, suggesting that thyroid disorders may potentially play a role in the development of OLP. However, it's important to acknowledge that our study has limitations due to the relatively small sample size.

Based on our findings, we recommend that individuals diagnosed with OLP, especially women, consider undergoing routine screening for thyroid disease as part of their healthcare regimen. Nevertheless, it is essential to emphasize that additional clinical research with larger sample sizes and more comprehensive evaluations is necessary to confirm and expand upon these conclusions. This will contribute to a better understanding of the complex relationship between OLP and thyroid disorders.

Ethical Approval

The study was conducted following the Declaration of Helsinki and approved by the Ethics Committee at the University of Prishtina "Hasan Prishtina," Pristina, Kosovo. All participants provided written informed consent.

Competing Interests

The authors declare that they have no competing interests.

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International Journal of Biomedicine 13(4) (2023) 317-322
http://dx.doi.org/10.21103/Article13(4)_OA14

ORIGINAL ARTICLE

Dentistry

INTERNATIONAL JOURNAL OF BIOMEDICINE

Effect of Preheating on Mechanical Properties of Different Commercially Available Dental Resin Composites

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Abstract

Background: This study aimed to reveal the effect of preheating on the surface microhardness and shear strength of composite materials used in the restoration of posterior teeth.

Methods and Results: There were 3 composite materials under the study: Estelite Posterior, Harmonize and Filtek Z550. To make static and dynamic tests of them, 120 filling samples were prepared. Of those, 60 samples were for surface hardness measurements and 60 samples were used to evaluate the shear strength of composite materials. We formed 12 study groups with 10 filling samples in each. Samples made off Estelite Posterior, Harmonize™, and Filtek™ were designated with E, H, and F capital letters, respectively; the «VH» abbreviation indicated static Vickers hardness testing and «SS» was assigned for dynamic shear testing; mark (°) was used when preheating was applied. Filling samples were made of heated (up to 60°C) and room-temperature (23-25°C) composite materials. The filling samples of EVH, E°VH, HVH, H°VH, FVH, and F°VH groups were subjected to a surface microhardness test. The samples of ESS, E°SS, HSS, H°SS, FSS, and F°SS groups were subjected to shear-strength assessment of materials. The surface microhardness of filling samples was measured using a ПМТ-3 Vickers hardness tester and the Vickers hardness number (VHN) was calculated. Dynamic tests were carried out using an UltraTester machine (Ultradent, Inc., USA) and shear test method until the shear-strength filling sample had completely failed.

After analysis of the obtained results, it was found that preheating had enhanced the surface hardness and mechanical strength of the composite materials used in the study. However, the positive influence of preheating was significant only in the EVH-E°VH, ESS-E°SS, HSS-H°SS, and FSS-F°SS groups in 1.48, 1.09, 1.33, and 1.16 times, respectively. In the HVH-H°VH and FVH-F°VH groups, the identified differences were not of significance despite the improvement in mean values at 1.1 and 1.1 times.

Conclusion: Preheating of light-curing resin-based composites is not equally effective for static and dynamic mechanical properties of materials for dental restoration. Preliminary laboratory tests could have helped before their clinical use.**(International Journal of Biomedicine. 2023;13(4):317-322.)**

Keywords: composite materials • preheating • Vickers hardness • shear strength

For citation: Melkumyan TV, Sheraliava SSh, Mendosa EYu, Khabadze ZS, Makeeva MK, Kamilov NKh, Musoshayhova ShK, Dadamova AD, Shakirov ShM, Mukhamedov AA. Effect of Preheating on Mechanical Properties of Different Commercially Available Dental Resin Composites. International Journal of Biomedicine. 2023;13(4):317-322. doi:10.21103/Article13(4)_OA14.

Abbreviations

VH, Vickers hardness; **VHN**, Vickers hardness number; **SS**, shear strength.

Introduction

The limited lifespan of tooth-colored composite restorations caused by their early failure is one of the pressing problems in contemporary dentistry. This situation is multifactorial and may arise from the technological subtleties in the manufacturing process up to the unfavorable interplay of monomers, fillers, and photoinitiators in the composition of restorative systems. Sometimes, direct restoration of teeth can be technically sensitive, not to mention individual characteristics of mandible biomechanics and even minor aberrations in occlusion or tooth position in the arch of each patient.[1-7]

Many commercially available composites already have excellent physical properties, chemical stability, and functional and cosmetic characteristics, allowing them to act as a good alternative to expensive ceramic restorations. However, the insufficient strength of resin-based materials is the subject of constant study to improve them.[8-10]

In this regard, for more than half a century, studies have searched for better monomers or mixtures. Also, great attention is paid to the size and shape of filler particles with silane-coupling agents and their total weight and volumetric load. In the end, the proper compositions for new materials with exceptional physical properties may be found and used in restoring teeth in areas of high occlusal load.[11,12]

It is known that the physicochemical properties of composite restoration largely depend on the quality of the polymer matrix, and the amount of residual bonds is highly influenced by light and thermal energy. It has also been noted that heating composite materials before photoactivation can increase their degree of conversion by reducing the viscosity of loaded polymers and increasing the mobility of free radicals. At the same time, reducing the percentage of remaining double bonds in composite restorations will help to improve their chemical stability and mechanical strength.[12-14]

On the contrary, the increase in the conversion degree of double bonds may also be accompanied by high values of polymer volumetric shrinkage, which may cause marginal gap formation and microleakage of restorations. Furthermore, the high rate of polymerization in heated resin-based composites during photoactivation may contribute to the formation of polymer stress, which in turn has a negative effect on the physical properties of the final restoration.[13-17]

Most resin-based materials available on the market mainly contain bisphenol-A-glycidyl methacrylate (Bis-GMA), urethane dimethacrylate (UDMA), triethylene glycol dimethacrylate (TEGDMA), and bisphenol-A-ethoxylated-glycidyl dimethacrylate (Bis-EMA). The properties of these monomers have been well studied separately and are not of particular interest. However, their mixtures are the subject of ongoing research.[18]

Besides the organic matrix, the strength of a composite filling is predetermined by the amount and size of filler particles. In particular, the improvement in the mechanical properties of composite materials with high filler load has been confirmed by the results of static and dynamic tests.[19]

The main objective of other studies was to assess the influence of filler particle shape on the shrinkage stress kinetics of composite resins during polymerization. It was found that the spherical shape is preferable and does not contribute to the occurrence of high shrinkage stress, compared to irregularly shaped particles. At the same time, the kinetics rate of polymer stress varies depending on the particle size of the dispersed phase.[20,21]

The amount of internal stress and its kinetics directly depend on the filling material's temperature during polymerization. Evidence indicates that the mechanical properties of dental restorative composites can be improved by increasing their degree of conversion. However, most of these data are based on the results of static trials, which cannot provide insight into the behavior of a composite restoration under dynamic loading, which usually occurs during the functional activity of the masticatory muscles.[22-26]

The main feature of static tests, in comparison to dynamic, is they do not lead to the destruction of specimens. The most common static trial used in dentistry is the assessment of surface hardness of fillings. However, to perform dynamic tests on composites, testing machines are necessary that can evaluate the resistance of the filling material to bending, compression, torsion, shear, etc.[25-28]

In this regard, this study aimed to reveal the effect of preheating on the surface microhardness and shear strength of composite materials used in the restoration of posterior teeth.

Materials and Methods

There were 3 composite materials under the study (Table 1). To make static and dynamic tests of them, 120 filling samples were prepared. Of those, 60 samples were for surface hardness measurements. They were cylindrical with a diameter of 5.5±0.05 mm and a height of 2.5±0.1 mm (Figure 1). The other 60 samples were made in the shape of circular rods with an average cross-sectional diameter of 2.47±0.05 mm and length of 8.23±0.1mm (Figure 2). They were used to evaluate the shear strength (SS) of composite materials.

Table 1.

Composite materials under the study

Composite Material	Basic Composition	Lot
Estelite Posterior (E)	Matrix: Bis-GMA, TEGDMA and Bis-MPEPP. Filler load SiO_2-ZnO_2 (84 wt%): mean particle size 2 μm (0.1-10 μm)	W1923
Filtek™ Z550 (F)	Matrix: Bis-GMA, UDMA, Bis-EMA, PEGDMA and TEGDMA. Filler load SiO_2-ZnO_2 (82 wt%): mean particle size 3 μm	NC54995
Harmonize™ (H)	Matrix: Bis-GMA, Bis-EMA and TEGDMA. Filler load SiO -ZnO_2 (81 wt%): particle size 0,05-400 μm	9768511

Bis-EMA - bisphenol-A-ethoxylated-glycidyl dimethacrylate; Bis-GMA - bisphenol A-glycidyl methacrylate, Bis-MPEPP - bisphenol A polyethoxy methacrylate, PEGDMA- polyethylene glycol dimethacrylate, TEGDMA - triethylene glycol dimethacrylate, UDMA- urethane dimethacrylate.

Fig. 1. *Filling samples for Vickers hardness evaluation*

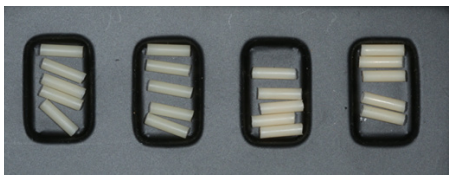

Fig. 2. Filling samples for shear strength assessments.

Thus, 12 study groups were formed with 10 filling samples in each. Samples made off Estelite Posterior, Harmonize™, and Filtek™ were designated with E, H, and F capital letters, respectively; the "VH" abbreviation indicated static Vickers hardness testing and "SS" was assigned for dynamic shear testing; mark (°) was used when preheating was applied.

Filling samples were made of heated (up to 60°C) and room-temperature (23-25°C) composite materials. Preheating was carried out on a calibrated appliance representing a heating glass tray (Figure 3). The design of the heater made it possible to polymerize composite materials at 60°C.

Fig. 3. *Appliance for heating dental composites.*

Photoactivation of the light-cured materials was carried out following the manufacturer's instructions using a VALO cordless curing light (Ultradent Products, Inc., USA) in standard mode.

Following the ISO 4049 protocol, after photoactivation, the prepared samples were immersed in water and stored at 37°C for 24 hours. Mechanical tests were carried out after this period of time.

The surface microhardness of filling samples was measured using a ПМТ-3 Vickers hardness tester. A 100-gram load was applied for 10 sec. In each sample of VH groups, 9 imprints were arbitrarily made on the top surface. The diagonals of square indentations were fixed in microns. Measurements were made on images (Figure 4) obtained using a scanning electron microscope SEM-EVO MA 15 (Zeiss, Germany). To get a clear image from the surface of the filling samples, they were sputtered with gold using a Q150R ES appliance (Quorum Technologies, UK).

The Vickers hardness number (VHN) was calculated according to the following formula: $VHN = 1.854 \times (F/D^2)$, where F is the applied load (measured in kilograms-force) and D^2 is the area of the indentation (measured in square millimeters) (Figure 4), which yields the VHN in the units of kg/mm^2.

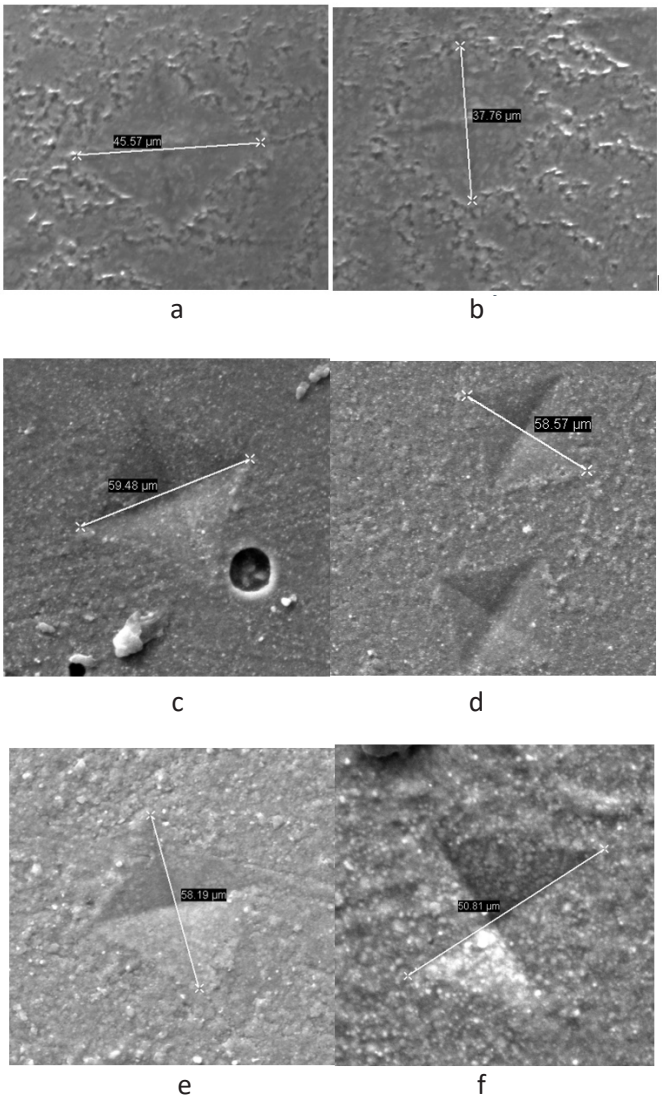

Fig. 4. *a - EVH; b - EºVH; c - HVH; d - HºVH; e -FVH; f - FºVH*

Dynamic tests were carried out using an UltraTester machine (Ultradent, Inc., USA) and shear test method until the shear-strength (SS) filling sample had completely failed. For this purpose, a steel adapter was made, which was fixed in the test clamp-base after it was mounted on the lifting platform.

The adapter resembled a barrel into which the shear-strength filling sample was inserted (Figure 5). The platform lifting speed was 0.1 mm/min. The peak load values were captured in pounds (lb).

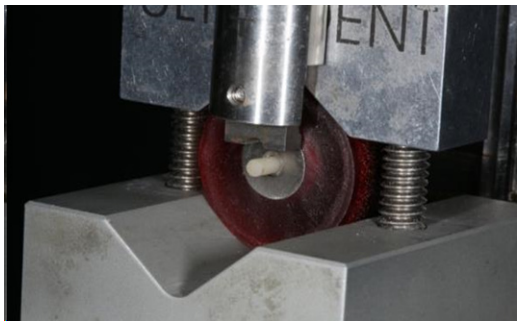

Fig. 5. *Steel adapter with inserted filling sample before the test.*

Statistical analysis was performed using the statistical software package SPSS version 21.0 (SPSS Inc, Armonk, NY: IBM Corp). For descriptive analysis, results are presented as mean±standard deviation (SD). The Mann-Whitney U Test was used to compare the differences between the two independent groups. A probability value of $P<0.05$ was considered statistically significant.

Results

The filling samples of EVH, EºVH, HVH, HºVH, FVH, and FºVH groups were subjected to a surface microhardness test. The samples of ESS, EºSS, HSS, HºSS, FSS, and FºSS groups were subjected to shear-strength assessment of materials. After analysis of the obtained results (Table 2), it was found that preheating had enhanced the surface hardness and mechanical strength of the composite materials used in the study. However, the positive influence of preheating was significant only in the EVH-EºVH, ESS-EºSS, HSS-HºSS, and FSS-FºSS groups in 1.48, 1.09, 1.33, and 1.16 times, respectively. In the HVH-HºVH and FVH-FºVH groups, the identified differences were not of significance despite the improvement in mean values at 1.1 and 1.1 times.

Table 2.

Influence of preheating on VH and SS of resin composites in vitro

Resin Composite	VHN (kg/mm^2)	Shear Strength (lb)
E *P-value* Eº	79.6 ± 15.3 0.000 118.1 ± 21.8	80.0 ± 5.4 0.002 87 ± 3.0
H *P-value* Hº	53.6 ± 7.3 >0.05 63.7 ± 15.0	54.2 ± 5.8 0.000 71.2 ± 4.5
F *P-value* Fº	73.8 ± 27.3 >0.05 87.5 ± 22.0	71.0 ± 4.9 0.000 82.5 ± 4.0

Discussion

Improving the quality of composite restorations is one of contemporary dentistry's main priorities. In this regard, the development of new materials and methods of their application will be relevant subjects for scientific research for many years.

It is known that preheating the composite material can significantly enhance the mechanical properties of restoration and its resistance to wear under masticatory load. In this regard, the positive results obtained from dynamic in vitro testing of the resin-based composite may shift the treatment plan strategy from an indirect approach to less invasive direct tooth-colored restoration.[27-31]

A diametrical load or split Hopkinson pressure bars are often used to evaluate the mechanical properties of composite dental resins under compressive load at different rates. According to them, a cylindrical sample is subjected to compression in a diametrical plane perpendicular to the longitudinal axis of the test sample.[26] This type of force distribution may closely simulate the incidence of stress encountered in Class 1 restorations. However, Class 2 composite restorations have a great chance of chipping the filling's mesial or distal occlusal margin due to the lack of supporting tooth wall and the risk of shear stress occurrence. As a result, the filling may fail when there is an occlusal load on one part of it and not on the other.[32]

Shear stress results from the action of forces directed at each other but in different planes.[33,34] In this regard, assessing the physical properties of materials by shear force in a cantilever system, rather than diametrically directed, could be more accurate, especially for the composites used in Class 2 cavity restoration.

Present research did not reveal all the subtleties of different behavior of composites in the study when the preheating approach was applied. However, certain observations were made which could be of particular value for daily practice.

For instance, the lack of a significant influence of preheating on surface microhardness of filling samples made off Harmonize™ and Filtek™ was presumably due to an insufficient filler load of their polymer matrix. This preliminary conclusion was drawn from similar tests performed with Estelite Posterior and its technical parameters, indicating heavier loading with SiO_2 and ZnO_2 nanoparticles, compared to other composites in the study.

On the contrary, a significant improvement in the shear strength of all studied materials after applying the preheating approach indicated an increased degree of conversion in them and no occurrence of significant internal stress, which was probably leveled out by the spherical shape of the filler particles.

Thus, the results of this study showed that applying a preheating approach for light-curing composites is not equally effective for static and dynamic mechanical properties of dental restorative materials, and that preliminary laboratory tests could have helped before their clinical use.

Conflict of Interests

The authors declare that they have no conflicts of interest.

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International Journal of Biomedicine 13(4) (2023) 323-328
http://dx.doi.org/10.21103/Article13(4)_OA15

ORIGINAL ARTICLE

Epidemiology and Population Health

INTERNATIONAL JOURNAL OF BIOMEDICINE

Demographic, Clinical, and Biomedical Profile of Diabetic Patients Receiving Home Healthcare in Saudi Arabia

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Abstract

***Background*:** Identifying characteristics of diabetic patients receiving home healthcare will help in designing services that respond to their conditions and improve their health status and quality of life. The aim of this study was to describe the demographic, clinical, and biomedical characteristics of diabetic patients receiving home healthcare (HHC).

Methods and Results: We used a descriptive cross-sectional design, and data were collected from 251 medical records of diabetic patients in two home healthcare centers in Saudi Arabia. The collected data included demographic, clinical, and biomedical profile variables. The average age was 74.7±11.6 years, with most patients (93.2%) aged 60 or older. The most common treatment modality was multiple daily insulin injections with or without oral medication (38.6%), followed by oral medication with sulfonylurea (19.9%). Pressure injury was the most reported complication/comorbidity, affecting 33.1% of patients. Cerebrovascular disease came next, affecting 20.7% of patients, followed by cardiovascular disease, ischemic heart disease, and nephropathy, affecting 12.3%, 10%, and 6.4% of patients, respectively. Only 4.2% of patients experienced hypoglycemia, and only 5.6% of patients were hospitalized due to diabetes mellitus complications. The mean HbA1c was 7.6±1.7%, with approximately 71.7% of the diabetic patients having HbA1c≤8%, and 28.2% with HbA1c>8% (P<0.0001). The median (range) low-density lipoprotein was 2.93 (1-317) mmol/L. The median (range) eGFR was 76.6 (9-389) mL/min/1.73m^2. Around 48% of the population had an eGFR<60 mL/min/1.73m^2.

Conclusion: Our findings show satisfactory glycemic control, acceptable LDL levels, low incidence of hypoglycemia, and minimal hospital admissions.**(International Journal of Biomedicine. 2023;13(4):323-328.)**

Keywords: diabetes mellitus • home healthcare • Saudi Arabia • glycemic control

For citation: Alzubaidi LM, Alsagheir AI, Hassanein MH, Khalifa RM, Luttfi IK. Demographic, Clinical, and Biomedical Profile of Diabetic Patients Receiving Home Healthcare in Saudi Arabia. International Journal of Biomedicine. 2023;13(4):323-328. doi:10.21103/Article13(4)_OA15

Abbreviations

CKD, chronic kidney disease; **DM**, diabetes mellitus; **eGFR**, estimated glomerular filtration rate; **HHC**, home healthcare; **HbA1c**, glycated hemoglobin; **LDL**, low-density lipoprotein; **SNDC**, Saudi National Diabetes Center; **SMBG**, self-monitoring of blood glucose; **T2D,** type 2 diabetes.

Introduction

Diabetes mellitus (DM) is a chronic disease that affects approximately 194 million individuals, equivalent to 5.1% of the adult population globally.[1] In Saudi Arabia, the age-adjusted prevalence of diabetes is currently estimated to be 18.7%, according to the International Diabetes Federation. This high prevalence places the country among the top five in the Middle East and North Africa region[2] and the top ten globally.[3]

DM can cause acute medical crises, such as diabetic ketoacidosis,[4] as well as long-term complications, including blindness, renal failure, and lower limb amputations.[1] These complications can cause significant suffering for individuals and have a considerable impact on their quality of life and overall health outcomes.[1,4]

To effectively prevent medical crises and long-term complications associated with diabetes, access to ongoing and timely care is crucial, and flexible follow-up schedules must also be in place to ensure comprehensive management of the disease.[4,5] However, the access and scheduling limitations associated with traditional clinic-based medical care might hinder the provision of such care.(5) Consequently, home-based care has emerged as a convenient alternative for the provision of diabetes management, particularly for elderly individuals and patients who are housebound or bed-dependent.

Home healthcare (HHC) is a recognized model of healthcare provision that encompasses a broad range of medical and health services delivered by skilled practitioners in the patient's home.[6,7] DM is currently listed behind congestive heart failure as the primary diagnosis for admission to HHC.[7,8] In Saudi Arabia, patients with DM are among the 17 categories that qualify for HHC services provided by the Ministry of Health (MoH).[9]

Numerous studies have evaluated the characteristics of diabetic patients in Saudi Arabia, including their glycaemic control levels.[3,10,11] However, there is a lack of data regarding diabetic patients who receive HHC. These patients may differ from the general diabetic population, as they may be older and experience mobility restrictions due to underlying conditions. Consequently, there is a pressing need to identify the profile of these patients to determine their current situation and to inform the development of tailored HHC services that enhance their health outcomes and overall quality of life. Therefore, the aim of this study was to describe the demographic, clinical, and biomedical characteristics of diabetic patients receiving HHC.

Materials and Methods

This was a descriptive cross-sectional study conducted in the King Salman Hospital and Medina Home Healthcare Center. In 2005, King Salman Hospital became the first hospital to start providing homecare services in the Riyadh region. Currently, the hospital has 390 patients receiving home healthcare; around 50% of them have DM. The Medina Home Healthcare Center was also established in 2005 and currently provides care to 581 patients, with approximately 86% of them diagnosed with DM. The scope of the services provided under the home healthcare in both centers covers diagnosis, management, and follow-up of patients. Both HHC centers belong to the Saudi MoH and provide HHC services according to the regulations of the General Directorate of HHC in the MoH.

We used anonymized data from the medical records of patients with a diagnosis of DM who were receiving HHC from King Salman Hospital and Medina HHC Center. The sample included records of all age groups and both sexes. We calculated the sample size assuming a confidence level of 95%, a 50% hypothesized frequency of outcome factor in the population, and a non-response rate of 5%. The resulting sample size of 251 was divided proportionally between the two HHC centers. From each center, the medical records of diabetic patients were selected using the simple random sampling technique.

We collected data using a structured, pre-tested data collection sheet. The demographic variables included patient's age and sex. The clinical profile variables included type of DM, anti-diabetic medications, DM complications/comorbidities, hospital admissions, self-monitoring of blood glucose (SMBG), and retinal examination. The biomedical profile variables included the most recent results of the following laboratory tests: glycated hemoglobin (HbA1c), low-density lipoprotein (LDL), and creatinine level.

Using the HbA1c values, the study population was divided into two categories (HbA1c<8% and HbA1c≥8%) according to the SNDC guidelines for elderly diabetic patients with co-morbidities.[12] The eGFR was calculated using age, sex, ethnicity, and creatinine level, and the population was grouped based on SNDC guidelines as eGFR <60 or ≥60 mL/min/1.73m^2.[12]

Statistical analysis was performed using the statistical software package SPSS version 21.0 (SPSS Inc, Armonk, NY: IBM Corp). Baseline characteristics were summarized as frequencies and percentages for categorical variables. For the descriptive analysis, results are presented as mean (M) ± standard deviation (SD) or as median and range (Min-Max). Group comparisons with respect to categorical variables were performed using chi-square test. A probability value of $P<0.05$ was considered statistically significant.

Ethical considerations

The research was considered exempt from review by the Institutional Review Board (IRB) of Prince Nourah bint Abdulrahman University (IRB registration number 22-1051). This study fully complies with the STROCSS 2021 criteria13.[13]

Results

Demographic and Medical Information

We collected data from 252 medical records of eligible diabetic patients with a response rate of 99.6%.

Table 1 presents the demographic information of the study population. The average age was 74.7±11.6 years, with most patients (93.2%) aged 60 or older. Females accounted for 65.3%. Most patients had T2D (Table 2). About half (50.2%) and three-quarters (75.7%) of the patients were bedridden or homebound, respectively. The most common treatment modality was multiple daily insulin injections with or without oral medication (38.6%), followed by oral medication with sulfonylurea (19.9%). About 16% of the patients were managed with a combination of oral anti-diabetic therapy

and basal insulin, and 11.6% were managed with metformin alone. Approximately 7% of patients were not taking any anti-diabetic medication. Most patients (95.2%) performed SMBG (Table 2).

Table 1.

The background characteristics of diabetic patients receiving home healthcare (n = 251)

	Mean (SD)	Median (Min-Max)
Age, years	74.7 (11.6)	75(31-107
	Frequency	Percent
Age groups		
<60	17	6.8
60 - 74	103	41.0
75 - 89	112	44.6
≥90	19	7.6
Sex		
Male	87	34.7
Female	164	65.3

Table 3.

Diabetes complications/comorbidities among diabetic patients receiving home healthcare (n = 251)

	Frequency	Percent
Complications/comorbidities		
Pressure injuries	83	33.1
Cerebrovascular disease	52	20.7
Cardiovascular disease	31	12.4
Ischemic heart disease	25	10
Nephropathy	16	6.4
Hypoglycemia	12	4.8
Peripheral neuropathy	5	2
Retinopathy proliferative and non-proliferative	4	2.4
Hearing impairment	3	1.2
Other	2	2.8
Readmission for complications		
Yes	14	5.6
No	237	94.4

Diabetes Complications/Co-morbidities

Pressure injury was the most reported complication/comorbidity, affecting 33.1% of patients. Cerebrovascular disease came next, affecting 20.7% of patients, followed by cardiovascular disease, ischemic heart disease, and nephropathy, affecting 12.3%, 10%, and 6.4% of patients, respectively (Table 3). Only 4.2% of patients experienced hypoglycemia, and only 5.6% of patients were hospitalized due to DM complications.

Table 2.

The medical profile of diabetic patients receiving home healthcare (n = 251)

	Frequency	Percent
Type of Diabetes Mellitus		
Type 1	7	2.8
Type 2	244	97.2
Bedbound		
Yes	126	50.2
No	125	49.8
Housebound		
Yes	190	75.7
No	61	24.3
Treatment		
Oral and basal	41	16.3
Metformin alone	29	11.6
Oral non-sulfonylureas	16	6.4
Multiple daily injection with/without oral medications	97	38.6
Oral medication with sulfonylureas	50	19.9
No medication	18	7.2
Self-monitoring of blood glucose		
Yes	239	95.2
No	12	4.8
Retinal eye examination (last year)		
Yes	7	2.8
No	244	97.2

Biomedical Profile

The mean HbA1c was 7.6±1.7%, with approximately 71.7% of the diabetic patients having HbA1c≤8%, and 28.2% with HbA1c>8% (P<0.0001). The median (range) LDL was 2.93 (1-317) mmol/L. The median (range) eGFR was 76.6 (9-389) mL/min/1.73m^2. Around 48% of the population had an eGFR<60 mL/min/1.73m^2 (Table 4).

Discussion

The demand for HHC services is increasing rapidly to cater to the needs of the growing elderly population and those suffering from chronic disorders.[6] This paper presents the population of patients with DM receiving HHC in Saudi Arabia.

Table 4.

The biomedical profile of diabetic patients receiving home healthcare .

	Frequency	Percent	*P*-value
HbA1c			
≤ 8%	180	71.7	<0.0001
> 8%	71	28.3	
eGFR			
<60 mL/min/1.73m^2	122	48.6	0.6586
≥ 60 mL/min/1.73m^2	129	51.4	
	Mean (SD)	Median (Min-Max)	
HbA1c	7.6(1.7)	7.3(5-14)	
LDL	11.28 (24.0)	2.93 (1-317)	
eGFR	84.2 (44.9)	76.6 (9-389)	

Background and Medical Information

Our results revealed that most of the study population were elderly, which is expected as the majority of HHC patients are 65 years of age and older.[14] On the other hand, while global patterns suggest that DM affects males more than females,[15] our study found a higher proportion of female patients. However, it is important to note that this gender disparity may be due to the higher demand for HHC services among females rather than a higher susceptibility to DM. As such, it is crucial to ensure the inclusion of female health workers as part of the HHC team.

Our study also revealed that a significant proportion of the study population were bedbound or housebound. These findings underscore the importance of home-based care for diabetic patients with disabilities who need ongoing and timely care to effectively manage their DM.[5]

Diabetes Management and SMBG

Our study revealed a high utilization of insulin alone or in combination with oral medications among the study population; this finding differs from previous studies conducted in Saudi Arabia, which reported a lower use of insulin and a higher use of oral hypoglycemic agents.[16] However, it is important to note that the selection of pharmacological agents for diabetes management is patient-centered, based on their medical condition (life expectancy and risk of hypoglycemia), the risk for side effects, and preferences. The global guidelines for T2D[17] and the SNDC recommend this individualized approach.[12]

For individuals with T2Dtreated with insulin, clinical guidelines suggest regularly monitoring their BG levels to titrate insulin dosage and avoid hypoglycemia.[17] This practice of SMBG was observed to be high among our study population. This finding contrasts with a previous study in Saudi Arabia that reported lower rates of SMBG among T2D patients treated with oral glucose-lowering drugs alone or in combination with insulin, who receive clinic-based care.[16] As reported by several studies, HHC can improve BG monitoring and diabetes self-care techniques, leading to fewer hospitalizations and emergency department visits.[5,18,19] HHC presents a unique opportunity to encourage homebound patients to take charge of their diabetes management.[8]

Diabetes Complications/Comorbidities and Biomedical Profile

Diabetes is associated with multiple complications/comorbidities that can impact its management and influence patient outcomes. Our study revealed pressure ulcers as the most prevalent complication/comorbidity among the study population. Due to their bedridden status, advanced age, and DM, our patients are particularly susceptible to pressure ulcers.[20,21] These findings underscore the significance of incorporating pressure ulcer prevention and management into diabetes care, with a focus on addressing the contributing factors that predispose patients to this condition.[20]

Our study also identified cerebrovascular disease, cardiovascular disease, and ischemic heart disease as complications/comorbidities among our patient population. However, the prevalence of cardiovascular and ischemic heart diseases in our study lies toward the lower limit of the range reported from other countries for coronary heart disease in diabetic patients (ranging from 1.8% to 43.4% in population-based studies).[1] The median LDL revealed by our study (2.9 (1-317) mmol/L) was slightly higher than the target of less than 2.6 mmol/L recommended by the Saudi National Dyslipidemia Committee.[12] Our finding is also consistent with another study conducted in Saudi Arabia, which reported a mean LDL level of 2.7±1.2 mmol/L among diabetic patients receiving clinic-based diabetes care.[3] This finding suggests that home-based care could be as effective as facility–based care for dyslipidemia management. However, there may still be room for improvement to reach the target set by the SNDC for LDL.

Additionally, our study identified nephropathy as one of the complications/comorbidities of DM in our patient population's medical records. However, upon calculating the eGFR in this study, we found a significant decline in this marker, indicating a higher prevalence of CKD than what was reported in the medical records. This finding is consistent with previous reports in Saudi Arabia, which suggest that DM is the leading cause of CKD.[12] In a previous study conducted in Saudi Arabia, more than half of the newly diagnosed patients in dialysis centers had T2D.[8] Our results underscore the importance of monitoring diabetic patients in the home setting for the early detection of CKD. It is crucial to increase efforts to identify and manage CKD among diabetic patients to prevent disease progression and improve their long-term outcomes.

Only a small proportion of our patients experienced hypoglycemia. This is in contrast to the results of other studies conducted in Saudi Arabia, which reported a prevalence of hypoglycemia as high as 12.5%[22] and 61.9%(23) among patients with T2D. The low prevalence of hypoglycemia in our population could be explained by the old age group and the high practice of SMBG.

The biomedical profile of our patients indicates a mean HbA1c of 7.6±1.7%, with the majority having an HbA1c level of ≤8%. This is a positive finding considering the population's functional dependency and multiple coexisting chronic illnesses. For a population with such characteristics, the SNDC recommends glycemic goals of HbA1c ranging from 8.0% to 8.5%.[12]

The average HbA1c level revealed by our study was lower than that reported in other studies targeting populations receiving facility-based diabetic care in Saudi Arabia. For example, Alshareef et al.[3] reported a mean HbA1c of 8.7±2.4%, while Alramadan et al.[10] reported a mean HbA1c of 8.5±1.9%. This suggests that home-based care may be an effective approach for diabetes management. Studies in many countries have shown that HHC provided to diabetic patients is effective in improving glycemic control.[5,19] In this context, our study revealed that a relatively small percentage of patients required hospital admission, suggesting that effective home healthcare services were provided to manage their conditions and prevent complications and exacerbations of the disease. This finding is consistent with the primary objective of home healthcare, which is to discharge patients to self or family care while minimizing the need for subsequent hospitalizations, particularly unplanned ones.[18]

Limitations of the Study

One of the limitations of this study was the use of secondary data, which may have limited the accuracy and completeness of the data collected. Another limitation is that the study was conducted in only two home healthcare centers in Saudi Arabia and may not be generalizable to other regions.

Conclusion

Our findings show satisfactory glycemic control, acceptable LDL levels, low incidence of hypoglycemia, and minimal hospital admissions. The biomedical profile of diabetic patients receiving HHC reveals a positive finding, with the majority having an HbA1c level of ≤8% and LDL median of 2.9 (1-317) mmol/L. However, eGFR level had declined, necessitating early detection and management of CKD. Pressure ulcers, cerebrovascular disease, cardiovascular disease, and ischemic heart disease were the most common complications/comorbidities among diabetic patients, requiring the inclusion of services targeting these conditions. The most common treatment modality was insulin with or without oral medication, and there is a high level of SMBG and a low hospital admission rate. Further studies are needed to assess the quality of care provided to diabetic patients in the home setting.

Competing Interests

The authors declare that they have no competing interests.

Acknowledgments

The authors are grateful to the staff of the homecare health centers for their contribution to the data collection.

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International Journal of Biomedicine 13(4) (2023) 329-333
http://dx.doi.org/10.21103/Article13(4)_OA16

ORIGINAL ARTICLE

Occupational Diseases

INTERNATIONAL JOURNAL OF BIOMEDICINE

Estimation of Pulmonary Functions and Cardiovascular Indices among Workers in Al-Samawa Oil Refinery

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Abstract

Background: Oil refinery employees suffer from exposure to fumes from petrol that contain harmful chemicals that can hurt the lungs and cardiovascular system. This research aimed to assess workers' health at the Al-Samawa oil refinery in Iraq, specifically their pulmonary function and cardiovascular wellness.

Methods and Results: Sixty workers at the Al-Samawa oil refinery, with a mean exposure duration of 8.7±4.23 years, were compared to sixty non-exposed healthy participants (the control group) of the same age, height, and weight.

Workers at the Al-Samawa oil refinery were divided into three subgroups of twenty each based on their work: production workers (PW), operation workers (OW), and technical workers (TW). Pulmonary function was evaluated using a spirometer, and blood pressure was recorded using a digital wrist cuff monitor. The current study demonstrated that there was a highly significant drop in the mean values of FVC, FEV_1, FEV_1/FVC%, PEFR, and $FEF_{25-75\%}$ of employees at the Al-Samawa oil refinery, in comparison with the control. According to our findings, the mean values of FVC and FEV1 among PW and OW were significantly lower than the control values. In addition, among OW and TW, FEV_1 and FEV_1/FVC significantly decreased, compared to control. Furthermore, the mean values of PEFR and $FEF_{25\%-75\%}$ in OW were significantly lower than the control. Mean FVC, PEFR, and $FEF_{25\%-75\%}$ values were significantly lower in OW than in PW and TW. Furthermore, the mean values of FEV1 and FEV1/FVC in OW were significantly lower than in PW. Systolic BP and diastolic BP were significantly higher in the Al-Samawa oil refinery workers than in the control.

Conclusion: The Al-Samawa oil refinery workers have impaired respiratory functions. In addition, the lung function of those working in the operations department was significantly lower than that of individuals working in other occupations. Exposure to petrol vapors in the workplace is associated with increased BP.**(International Journal of Biomedicine. 2023;13(4):329-333.)**

Keywords: pulmonary functions • blood pressure • air pollution • petrol

For citation: AL-Hakkak ZMM. Estimation of Pulmonary Functions and Cardiovascular Indices among Workers in Al-Samawa Oil Refinery. International Journal of Biomedicine. 2023;13(4):329-333. doi:10.21103/Article13(4)_OA16

Abbreviations

BMI, body mass index; **BP**, blood pressure; **DBP**, diastolic BP; **FEF**, forced expiratory flow; **FVC**, forced vital capacity; **FEV_1**, forced expiratory volume in the first second; **OW**, operation workers; **PM**, particulate matter; **PCBs**, polychlorinated biphenyls; **PAHs**, polycyclic aromatic hydrocarbons; **PEFR**, peak expiratory flow rate; **PW**, production workers; **SBP**, systolic BP; **TW**, technical workers.

Introduction

Air pollution continues to get much attention worldwide because it hurts people's health and well-being. Some diseases and conditions, such as asthma, chest pain, lung cancer, loss of breath, and sore throat, have been linked to air pollution.[1]

Oil refinery employees suffer from exposure to fumes from petrol that contain harmful chemicals that can hurt the lungs and cardiovascular system. Petrol is dangerous because it contains various toxic chemicals, including volatile aliphatic and aromatic hydrocarbons known as BTEX (benzene, toluene, ethylbenzene, and xylene.[2] Although oil refineries are the primary producers of volatile aromatic hydrocarbon emissions, they generate excessive air, water, and land pollution in the surrounding areas.[3] Refineries and petroleum plants are the most prominent industries that release volatile organic compounds.[4] Refineries and petrochemical companies generate many kinds of harmful pollutants that are

released into the environment. These include volatile organic compounds, metals, polycyclic aromatic hydrocarbonss, and polychlorinated biphenyls. Wheezing and other symptoms of respiratory diseases were found to have a higher prevalence in the data collected from locations close to petrochemical industries.[5]

Refinery employees are especially at risk from chemical pollution because they inhale high amounts of benzene.[6] In hot countries like Iraq, oil refinery workers are more likely to be exposed to benzene gas, raising stress about their health. Higher atmospheric temperatures make it more probable that one will inhale or absorb these dangerous pollutants through the skin.[7] In addition, refined petroleum compounds are prevalent and have been shown to harm several human biological systems.[8] Residents who were exposed to petrochemical pollution reportedly had respiratory problems.

The human body reacts differently to these chemical molecules, and one impact is the provocation of several occupational cancers.[9] Several human and animal investigations have shown that the exhaust emissions from petrol, kerosene, and petrol include chemical compounds like cadmium, benzene, and volatile nitrates that are toxic to bone marrow, the lymph nodes, and the spleen.[10] Pollutants from petroleum products have been observed to alter liver enzyme levels and the production of hormones in the pituitary gland.[11] There is a greater possibility that refinery workers might get asthma, chronic bronchitis, or COPD because of their jobs.[12] Volatile organic compounds have a wide variety of adverse short-term and long-term effects on human health, some of which include irritation of the respiratory tract, digestive problems, dysfunction of the central nervous system, narcosis, effects on growth, and carcinogenicity.[13]

Air pollution has been linked to cardiovascular illnesses, including arrhythmias, ischemia, infarction, atherosclerosis, and hypertension.[14] Exposure to CO, PM2.5, and PM10 increases the risk of raised blood pressure (BP).[15] Multiple studies have found that exposure to NO_2, SO_2, metals, PM10, O_3, and polycyclic aromatic hydrocarbons increases the risk of lung and cardiac disease, eye irritation, and coughing.[16,17] Exposure to vehicle pollution for a long time affects lung function, elevates BP, reduces immunity, and increases the chance of developing lung cancer.

The findings of this study will assist the Al-Samawa oil refinery in evaluating its workers' health and taking steps to enhance the daily lives of those working in the oil sector. Few studies have investigated cardiovascular indices and lung function tests in oil refinery workers. Therefore, this research aimed to assess workers' health at the Al-Samawa oil refinery in Iraq, specifically their pulmonary function and cardiovascular wellness.

Materials and Methods

A cross-sectional design was selected for the study. This research was carried out at the oil refinery at Al-Samawa, which is in the southern Al-Muthanna province of Iraq, approximately 280km southeast of Baghdad. The refinery was mainly owned by the Midland Refineries company of the Ministry of Oil in the Republic of Iraq. The period for data collection was from December 2022 to February 2023.

The employees at the refinery face direct and indirect danger due to the pollutants in the air in the workplace. Sixty workers at the Al-Samawa oil refinery, with a mean exposure duration of 8.7±4.23 years, were compared to sixty non-exposed healthy participants (the control group) of the same age, height, and weight (Table 1). Workers at the Al-Samawa oil refinery were divided into three subgroups of twenty each based on their work: production workers (PW), operation workers (OW), and technical workers (TW). Pulmonary function was evaluated using a spirometer, and BP was recorded using a digital wrist cuff monitor. Employees in the three exposure groups and the control had no previous illnesses related to their respiratory, allergic, or cardiovascular systems. They worked six days a week, for 8-10 hours a day.

BMI (kg/m^2) was calculated by measuring the employees' and controls' heights and weights on a properly calibrated scale.[18] All the lung function measures were carried out at the participant's place of employment by a portable Spirolab III, a computerized diagnostic spirometer (MIR, Rome, Italy).[19] The individual was seated throughout the examination, and a nose clip was used to place a mouthpiece in his mouth after it had been firmly ringed by his lips. The participants in the test were always given instructions that were very clear and accurate, and those instructions were to inhale as deeply as possible and then to expel as forcefully and quickly as possible into the mouthpiece. The test was administered three times, each following an acceptable rest period, and the findings were obtained using a spirometer.[20] FVC, FEV1, FEV1/FVC ratio, FEF, and $FEF_{25-75\%}$ were recorded.

Table 1.

Physical characteristics of Al-Samawa oil refinery workers compared to physical characteristics of control group.

Variable	Control (n=60)	Oil refinery workers (n=60)	*P*-value
Age, yrs	37.90 ± 6.16	38.64 ± 6.12	0.551
Weight, kg	76.44 ± 9.84	77.52 ± 9.52	0.542
Height, cm	171.94 ± 5.37	172.16 ± 5.34	0.822
BMI, kg/m^2	25.90 ± 2.81	26.16 ± 2.86	0.616

Workers at the Al-Samawa oil refinery got their BP measured using a computerized wrist cuff monitor. Two measures of the individual's BP were taken during one working day, with the average of those two readings recorded after each measurement.

Statistical analysis was performed using the statistical software package SPSS version 21.0 (SPSS Inc, Armonk, NY: IBM Corp). For the descriptive analysis, results are presented as mean (M) ± standard deviation (SD). For data with normal distribution, inter-group comparisons were performed using Student's t-test. Multiple comparisons were performed with one-way ANOVA and Tukey HSD post-hoc test. A value of $P<0.05$ was considered significant.

Results

The current study demonstrated that there was a highly significant drop in the mean values of FVC, FEV1, FEV1/FVC%, PEFR, and $FEF_{25-75\%}$ of employees at the Al-Samawa oil refinery, in comparison with the control (Table 2).

Table 2.

Lung function parameters in oil refinery workers and the control group.

Parameter	Control group	Oil refinery workers	*P*-value
FVC (L)	4.60 ± 0.37	3.98 ± 0.62	<0.0001
FEV_1 (L)	3.71 ± 0.29	3.09 ± 0.38	<0.0001
FEV1/FVC (%)	87.60 ± 3.16	74.90 ± 3.36	<0.0001
PEFR (L/s)	6.82 ± 1.53	5.68 ± 1.29	<0.0001
$FEF_{25\%-75\%}$ (L/s)	3.87 ± 1.77	3.30 ± 1.25	0.0438

According to our findings, the mean values of FVC and FEV_1 among PW and OW were significantly lower than the control values (Table 2). In addition, among OW and TW, FEV_1 and FEV_1/FVC significantly decreased, compared to control. Furthermore, the mean values of PEFR and $FEF_{25\%-75\%}$ in OW were significantly lower than the control. Mean FVC, PEFR and $FEF_{25\%-75\%}$ values were significantly lower in OW than in PW and TW. Furthermore, the mean values of FEV_1 and FEV_1/FVC in OW were significantly lower than in PW (Table 3).

Table 3.

Lung function parameters in the subgroups of oil refinery workers.

Variable	Control group (1)	Oil refinery workers			Statistics One-way ANOVA and Tukey HSD post-hoc test
		PW (2)	OW (3)	TW (4)	
FVC (L)	4.53 ±0.39	3.94 ±0.29	3.58 ±0.71	4.35 ±0.27	F=28.3672, P=0.0000 P_{1-2}=0.0000, P_{1-3}=0.0000, P_{1-4}=0.3726, P_{2-3}=0.0457, P_{2-4}=0.0167, P_{3-4}=0.0000
FEV_1 (L)	3.67 ±0.33	3.21 ±0.29	2.73 ±0.74	3.10 ±0.57	F=24.3087, P=0.0000 P_{1-2}=0.0010, P_{1-3}=0.0000, P_{1-4}=0.0000, P_{2-3}=0.0070, P_{2-4}=0.8742, P_{3-4}=0.0590
FEV_1/FVC (%)	81.54 ±5.16	81.26 ±4.19	68.80 ±4.85	65.88 ±5.95	F=69.1823, P=0.0000 P_{1-2}=0.9965, P_{1-3}=0.0000, P_{1-4}=0.0000, P_{2-3}=0.0000, P_{2-4}=0.0000, P_{3-4}=0.2746
PEFR (L/s)	6.81 ±1.46	5.83 ±0.81	4.44 ±0.64	6.05 ±1.02	F=20.2432, P=0.0000 P_{1-2}=0.0102, P_{1-3}=0.0000, P_{1-4}=0.0714, P_{2-3}=0.0020, P_{2-4}=0.9373, P_{3-4}=0.0002
$FEF_{25\%-75\%}$ (L/s)	3.87 ±0.79	3.55 ±0.74	2.18 ±1.27	3.54 ±1.34	F=14.8800, P=0.0000 P_{1-2}=0.5893, P_{1-3}=0.0000, P_{1-4}=0.5643, P_{2-3}=0.0001, P_{2-4}=1.0000, P_{3-4}=0.0002

SBP and DBP were significantly higher in the Al-Samawa oil refinery workers than in the control (Table 4).

Table 4.

Blood pressure in oil refinery workers and control group.

Blood pressure	Control group	Oil refinery workers	*P*-value
SBP, mmHg	122.89 ± 3.74	128.33 ± 8.57	<0.0001
DBP, mmHg	81.17 ± 2.04	89.17± 4.92	<0.0001

Discussion

According to the findings, the employees at the Al-Samawa oil refinery had a significant decrease in FVC, FEV_1, FEV_1/FVC%, $FEF_{25\%-75\%}$, and PEFR compared to the control. These results corroborated the findings of Sajid et al.,[21] which showed that workers exposed to petrochemical compounds have a reduction in pulmonary function. Acute exposure to a polluted environment also changes lung functioning, as mentioned in Kodidala et al.[22] Additionally, the decrease in lung function measurements in oil refinery workers, compared to control, may indicate airway irritation from gaseous pollutants due to increased mucus production from goblet cells.

The research by Tanyanont et al.[23] was carried out in Thailand to investigate the effects of volatile organic compounds on the respiratory systems of people living near petrochemical production. Wheezing and other upper respiratory symptoms were more prevalent in samples from people close to petrochemical industries. BTEX contains monoaromatic hydrocarbons, which have been linked to severe health effects, such as asthma, nausea, and nasal, oral, and eye irritation.[24] Ramirez et al.[25] observed that individuals who live near refineries and are engaged in oil spill cleanup efforts suffered from several health issues, including skin, eye, and mucous membrane irritation, in their study on the pollution produced by crude oil extraction-refining and their impacts on human health.

Long-term contact with petroleum products significantly affects some people's lung function.[26] Some research shows that breathing in BTEX-H[n-hexane] can lead to adverse lung effects, such as asthma.[27] In an Italian study, SO_2 from oil refineries was highly linked to decreased lung function and increased airway inflammation.[28]

Similar to our results, Meo et al.[29] noticed that employees of oil refineries had significantly decreased lung function measurements, compared to their matched controls.

Workers in the Al-Samawa oil refinery operations department may have been exposed to a higher concentration of air pollutants than those in other departments, as measured by the low values of pulmonary function parameters. The petrochemical company generates several compounds from hydrocarbons and other petroleum refining products. Long-term exposure to petrochemical compounds and their vapors has been related to several diseases, including lung damage.[30]

Exposure to gases produced by refineries, power stations, and other factories has been linked to an increased risk of developing various illnesses, including cancer, heart disease, pneumonia, and mortality at an earlier age.[31] The acute and chronic toxicity of volatile organic compounds includes symptoms such as eye, nose, and throat irritation, headaches, nausea, dizziness, fatigue, and shortness of breath.[32] It has been established that emissions from petroleum refineries contribute to diseases, illnesses, and even mortality in persons close to petrochemical factories or petroleum refineries.[33]

SBP and DBP were significantly higher in the Al-Samawa oil refinery workers than in the control. Darwish et al.[34] stated that among the Aden refinery company workers, allergic conditions were the most frequently observed disease. Furthermore, hypertension was the second most common ailment (42.2%). Therefore, it is possible that this finding can be related to the observation that workers of the Al-Samawa oil refinery are significantly exposed to gaseous pollutants in their work environment, which causes vasoconstriction in the arterioles and increased vascular resistance, which ultimately leads to an elevation in BP. Increases in BP in fuel station workers have been attributed to the pressure effects of petrol fumes or particles.[35] Petroleum products should be recognized as possibly contributing to cardiovascular problems.

Polycyclic aromatic hydrocarbons, sulfur oxides, particulate matters, volatile organic compounds, polychlorinated biphenyls, nitrogen oxides, and O_3 are all air pollutants linked to an increased risk of cardiovascular illness.[36] A study of workers at Italian petroleum, gas, and energy companies found that those age 45 and above had a significantly greater risk of hypertension, hyperglycemia, and lipidemia.[37]

Conclusion

The current study has demonstrated significant declines in pulmonary function parameters in oil refinery workers exposed to petroleum refinery products in the workplace. This study concludes that the Al-Samawa oil refinery workers have impaired respiratory functions. In addition, the lung function of those working in the operations department was significantly lower than that of individuals working in other occupations. Exposure to petrol vapors in the workplace is associated with increased BP.

Ethical Considerations

The study protocol was reviewed and approved by the Ethics Committee of the University of Kufa**.** Written informed consent was obtained from all the participants.

Competing Interests

The authors declare that they have no competing interests.

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International Journal of Biomedicine 13(4) (2023) 334-340
http://dx.doi.org/10.21103/Article13(4)_OA17

ORIGINAL ARTICLE

Mental Health

INTERNATIONAL JOURNAL OF BIOMEDICINE

Graduate Medical Students' Mental Health Concerns During COVID-19 Pandemic

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Abstract

Medical students are more likely than the general population to experience perceived stress during the pandemic due to many variables. This study aimed to evaluate the stress levels and prevalence of different mental health conditions among graduate medical students in Al Kharj City. An anonymous online survey was conducted among graduate medical students of Prince Sattam bin Abdulaziz University (PSAU). For this investigation, the following scales were used to measure the prevalence of common mental health issues: DSM-5 Self-Rated Level 1 Cross-Cutting Symptom Measure-Adult (CCSM-A); Perceived Stress Scale (PSS-10-C), to measure COVID-19-related student stress; and the COVID-19 Student Stress Questionnaire to get the global stress score (GSS). Two hundred twenty-one students were contacted, and 214(96.8%) consented to participate in the study. According to the CCSM-A scale, anxiety (73%) and depressive symptoms (71%) were the most frequently reported symptoms by the students. After correcting for age and self-perceived COVID-19 risk, there was a significant relationship between anger, suicidal ideation, and substance use, on one hand, and the study year on the other graduate medical students who have mental health issues bear a heavy load. In the post-pandemic recovery period, regular mental health assessments and providing early and adequate mental health assistance to needy people are imperative.**(International Journal of Biomedicine. 2023;13(4):334-340.)**

Keywords: medical students • COVID-19 • mental health

For citation: Ali AHA, Alrudian N, Alqahtani MS, Alanazi SE, Alotaibi AZ, Almazroua AM, Abdalkareem MIA, Althubiti TA, Alanazi BG. Graduate Medical Students' Mental Health Concerns During COVID-19 Pandemic. International Journal of Biomedicine. 2023;13(4):334-340. doi:10.21103/Article13(4)_OA17

Introduction

The COVID-19 outbreak in KSA and related lockdowns have severely disrupted people's daily lives and negatively impacted their mental health. Lockdown measures like school and college closures have generally impacted education. Similarly, higher education has also been affected, especially medical programs. The education supply has continued in KSA through other channels, including online learning and instructional materials sent to medical students throughout the pandemic.[1] Medical students' mental health is an issue because they are more likely than non-medical students to experience depression, anxiety, and burnout, which they experienced even before the COVID-19 pandemic.[2-4] These increased risks have

been linked to several stressors associated with medical school training, including academic pressures; working in a demanding clinical setting where doctors are exposed to ethical dilemmas, death, and suffering; as well as the support and culture of the medical school. This includes the unspoken rule that illness is a sign of weakness and that doctors should be strong.[5] Medical education requires extensive study and training compared to many other academic fields; this may impact the student's mental health. Medical students' emotional, physical, and spiritual health is compromised by the numerous obligations and demands they must meet. Previous studies have revealed that the prevalence of identified mood disorders, suicidal thoughts, and psychological distress was much higher in medical students.[6] Due to their numerous added pressures, medical students are at a higher risk of experiencing stress during the COVID-19 pandemic. That includes the pupils' ongoing elevated risk of developing COVID-19 infection and the ensuing risk of spreading the virus to family members. Additionally, increased strain is brought on by the intense competition for admission to medical schools, adaptation to the more recent online teaching method, uncertainties surrounding examinations, and clinical postings.[7]

According to the current literature, medical students are more likely to experience stress, and it is important to determine their level of stress and its contributing factors. Considering this, this study's objectives were to determine the incidence of common mental health symptoms across psychiatric disorders and to measure stress levels during the COVID-19 pandemic among medical students in Al Kharj.

Materials and Methods

The study was launched at Prince Sattam bin Abdulaziz University (PSAU) in Al Kharj in the 2022-2023 academic year. A cross-sectional online survey of PSAU medical students from all years was intended for this study. All medical school students were eligible to participate. The ethical review board of the institution gave its prior approval (SCBR-120-2023). Students who declared an anxiety or depression diagnosis were subject to exclusion criteria.

Google Forms was employed to collect data and to create self-administered surveys for the study. To prevent repeated submissions for the study's completion, participants must be signed into their Google accounts when filling out Google Forms. Participants' email addresses were kept private to ensure confidentiality. All PSAU medical students were invited to participate in this survey during their first semester. The university's internet portal was used to distribute the survey. We began with second-year students, and participants ranged from second to fifth year. The university oversees the first year as the preparatory year through a single program. The online survey had a voluntary participation period that ended on May 20, 2023. The following data was gathered for the study: first, sociodemographic details about the participants and symptoms to evaluate mental health domains crucial for all psychiatric diagnoses; second, data on students' subjective levels of personal stress and sources of stress connected to COVID-19.

The Perceived Stress Score (PSS-10-C), a self-reported scale to measure the global level of perceived stress, and DSM-5 Self-Rated Level 1 Cross-Cutting Symptom Measure-Adult (CCSM-A) were employed. In addition, we used the COVID-19 Student Stress Questionnaire, a 7-point scale that yields a global stress score (GSS). CCSM-A, PSS, and GSS were analyzed according to the procedures outlined in the scales.[8,9] By the medical-school year, the percentage of participants with above-threshold domain scores on the CCSM-A instrument, PSS, and GSS were separated. According to the CCSM-A scale, adjusted odds ratios were analyzed to find the determinants of common mental health domains. Similarly, crude and adjusted beta coefficients (using linear regression) were performed to identify the predictors of PSS and GSS.

Statistical analysis was performed using the statistical software package SPSS version 26.0 (SPSS Inc, Armonk, NY: IBM Corp). Baseline characteristics were summarized as frequencies and percentages for categorical variables and as mean (M) ± standard deviation (SD) for continuous variables. A multiple logistic regression analysis was conducted to calculate the unadjusted and adjusted odds ratios (UOR and AOR) with 95% confidence intervals (95%CI). A value of $P<0.05$ was considered significant.

Results

Out of the 221 people who were contacted, 214 agreed to participate in the survey (a response rate of 96.8%). The participants' average age was 22.4±2.9 years. Second-year students made up the largest percentage of the participant cohort (27.6%), followed by third-year students (27.1%), fourth-year students (24.8%), and fifth-year students (20.5%). Every student, except four, had received their complete doses of the COVID vaccine. However, almost one-fifth of the 43(20.1%) students had a high self-perceived risk of COVID-19 transmission (Table 1).

Table 1.

Sociodemographic characteristics of the participants (n=214)

	n	Percentage
Age in years of the study participants, M±SD	22.4 ± 2.9	
Year of medical school		
Second	59	27.6 %
Third	58	27.1 %
Fourth	53	24.8 %
Fifth	44	20.5 %
High self-perceived risk of COVID-19 transmission	43	20.1%
Fully vaccinated for COVID-19	210	98.1 %

The distribution of participants who reported mild or severe CCSM-A symptoms and some illustrations of the questions are presented in Table 2. Table 3 shows the GSS distribution. The CCSM-A results, using the multivariate logistic regression method, connected to students' year in PSAU, are presented in Table 4.

Table 2.

Distribution of participants experiencing mild or severe symptoms of the CCSM-A tool (n=214). Below are example questions.

Participants' symptoms	n	Percentage
Less interest or pleasure in performing activities		
a	38	17.8
b	54	25.2
c	62	29.0
d	38	17.7
e	22	10.3
Feeling down, depressed, or hopeless		
a	46	21.5
b	63	29.4
c	42	19.6
d	33	15.4
e	30	14.0
Sleeping fewer hours than usual, but though have plenty of energy		
a	66	30.8
b	56	26.2
c	52	24.3
d	26	12.1
e	14	6.5
Feeling panic or being frightened		
a	74	34.6
b	54	25.2
c	48	22.4
d	24	11.2
e	14	6.5
Unexplained pains and aches (e.g., back, head, abdomen, joints, legs)		
a	104	48.6
b	30	14.0
c	44	20.5
d	22	10.3
e	14	6.5
Thoughts of actually hurting yourself		
a	150	70.09
b	28	13.08
c	26	12.1
d	8	3.7
e	2	0.9
Perceive sound, i.e., not audible to people around, for instance, voices, even while no one was in the vicinity		
a	175	81.8
b	22	10.3
c	14	6.5
d	2	0.93
e	1	0.47

Table 2 (continued).

Distribution of participants experiencing mild or severe symptoms of the CCSM-A tool (n=214). Below are example questions.

Participants' symptoms	n	Percentage
Difficulties with sleep that disturbed your quality of sleep over all		
a	92	43.0
b	36	16.8
c	25	11.7
d	32	15.0
e	29	13.5
Troubles with memory (e.g., acquiring new information) or with locality (e.g., locating your way to home)		
a	96	44.8
b	54	25.2
c	32	15.0
d	24	11.2
e	8	3.7
Experiencing powered to perform specified mental acts or behaviours over and over again		
a	128	59.8
b	32	15.0
c	28	13.1
d	16	7.4
e	10	4.7
Not knowing who you really are or what you want out of life		
a	126	58.9
b	32	15.0
c	30	14.0
d	14	6.5
e	12	5.6
Not feeling dear to other people or liking your relations with them		
a	96	44.8
b	34	15.9
c	32	15.0
d	28	13.1
e	24	11.2
Smoking any type of tobacco, i.e., a cigar, cigarette, or pipe, or chewing or snuffing tobacco		
a	166	77.6
b	18	8.4
c	14	6.5
d	4	1.9
e	12	5.6
Taking any of the undermentioned medicines ON YOUR OWN, i.e., without a prescription from a doctor		
a	182	85.0
b	15	7.0
c	10	4.7
d	6	2.8
e	1	0.47

a) Not at all; b) Occasional; less than a day or two;
c) Numerous days; d) More than half the days; e) Almost every day

Table 3.

Distribution of participants COVID-19 related Global Stress Score (n=214)

Perception during COVID-19 pandemic	n	Percentage
Perception of the possibility of contamination through his time pandemic		
a	70	32.7
b	72	33.6
c	48	22.4
d	18	8.4
e	6	2.8
Perception of the circumstances of social isolation imposed during this time of pandemic		
a	80	37.4
b	50	23.4
c	52	24.3
d	22	10.3
e	10	4.6
Perception of the associations with your relatives during this time of pandemic		
a	88	41.1
b	66	30.8
c	36	16.8
d	14	6.5
e	10	4.6
Perception of the rapport with colleagues at the university at the time of pandemic		
a	84	39.2
b	64	29.9
c	46	21.5
d	14	6.5
e	6	2.8
Perception of your relations with your university professors during time of COVID-19		
a	90	42.1
b	54	25.2
c	56	26.2
d	6	2.8
e	8	3.7
Perception of academic knowledge during time of COVID-19 pandemic		
a	64	29.9
b	62	29.0
c	44	20.5
d	24	11.2
e	20	9.3
Perception of the changes due to the social isolation in your sexual life during the period of COVID-19 pandemic		
a	156	72.9
b	26	12.1
c	24	11.2
d	7	3.3
e	1	0.47

a) Not at all stressful; b) Somewhat stressful; c) Moderately stressful; d) Very stressful; e) Extremely stressful

Symptoms of anxiety (73%) and depression (71%) were the two most prevalent among the various symptom measurements. The fifth-year medical students showed above-average levels of anxiety (88%) and depressive symptoms (88%) across all study years. The fourth-year students displayed the most anger. Throughout all study years, substance abuse, psychosis, and feeling alienated were the least common symptoms.

According to the PSS, approximately 65% of fifth-year students experienced significant stress, compared to 21% of other students (Figure 1). The mean PSS was 18.3±5.7, with third-year students having the lowest mean (16.7) and fifth-year students having the highest mean (22.4). In the unadjusted analysis, no statistically significant relationship existed between PSS and year of study.

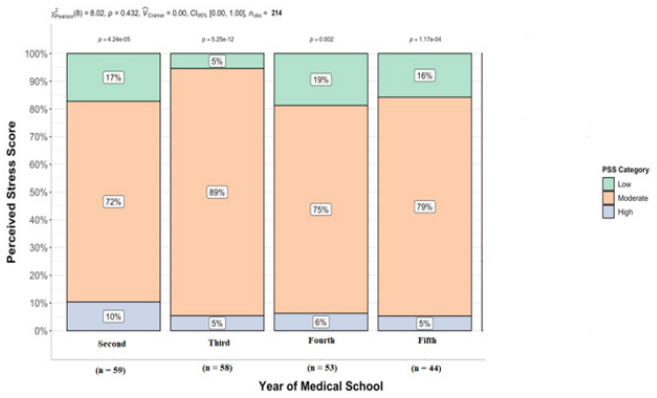

Fig. 1. *Distribution of participants' PSS categories by the year of medical school.*

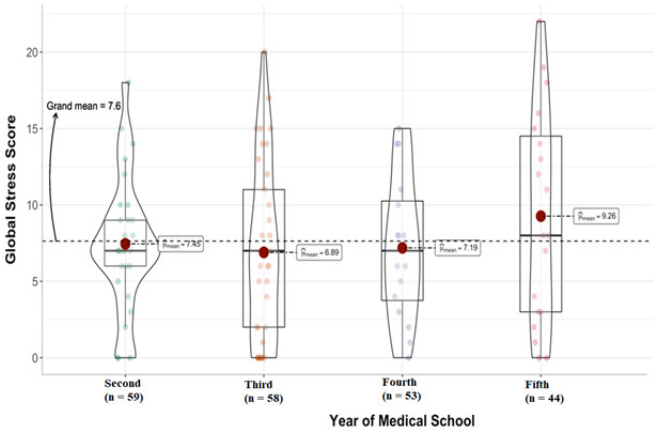

Fig. 2. *Distribution of GSS by year of medical school.*

Table 4.

Scores on CCSM-A associated with students' year in PSAU (multiple logistic regression analysis).

Symptoms	Second year	Third year	Fourth year	Fifth year	Age		High self-perceived risk of COVID-19	
					UOR	AOR	UOR	AOR
Depression	1.12 (0.28 – 3.99)	1.66 (0.29 – 6.28)	1.77 (0.40 – 8.66)	0.49 (0.12 – 4.31)	1.08 (0.89 – 1.32)	1.07 (0.85 – 1.35)	1.20 (0.46 – 3.16)	1.50 (0.52 – 4.36)
Anger	0.49 (0.23 – 1.44)	0.49 (0.43 – 1.77)	0.22 (0.11 – 0.79)	0.31 (0.19 – 1.55)	0.96 (0.82 – 1.11)	1.06 (0.87 – 1.29)	1.00 (0.40 – 2.57)	1.27 (0.45 – 3.59)
Mania	0.78 (0.33 – 2.99)	0.89 (0.21 – 3.88)	1.23 (0.21 – 8.11)	0.43 (0.14 – 3.99)	1.24 (1.00 – 1.53)	1.38 (0.93 – 2.04))	1.21 (0.49 – 3.01)	1.78 (0.65 – 4.89)
Anxiety	1.00 (0.31 – 4.74)	1.23 (0.33 – 5.00)	1.77 (0.44 – 8.11)	0.43 (0.15 – 5.11)	1.04 (0.87 – 1.25)	1.06 (0.86 – 1.32)	0.80 (0.28 – 2.25)	1.11 (0.37 – 3.31)
Somatic symptoms	0.99 (0.45 – 2.99)	0.86 (0.31 – 4.23)	1.23 (0.41 – 5.32)	0.85 (0.21 – 5.11)	1.03 (0.89 – 1.18)	1.02 (0.88 – 1.19)	1.22 (0.48 – 3.11)	1.26 (0.48 – 3.32)
Suicidal ideation	0.34 (0.17 – 0.88)	0.67 (0.32– 3.32)	0.29 (0.23 – 1.44)	0.34 (0.17 – 4.11)	1.05 (0.91 – 1.21)	1.10 (0.91 – 1.35)	0.44 (0.17 – 1.12)	0.39 (0.14 – 1.09)
Psychosis	0.89 (0.32 – 3.41)	0.79 (0.32 – 4.11)	0.79 (0.32 – 2.99)	0.59 (0.87 – 499)	1.10 (0.94 – 1.30)	1.17 (0.92 – 1.48)	0.46 (0.18 – 1.19)	0.64 (0.23 – 1.78)
Sleep problems	1.22 (0.43 – 4.23)	0.59 (0.22 – 3.00)	1.14 (0.39 – 3.99)	0.81 (0.22 – 3.99)	1.06 (0.91 – 1.22)	1.07 (0.89 – 1.28)	1.54 (0.60 – 3.99)	1.65 (0.61 – 4.48)
Substance use	0.14 (0.04 – 0.85)	0.25 (0.05 – 1.32)	0.19 (0.05 – 0.87)	0.33 (0.05 – 2.43)	0.90 (0.72 – 1.22)	1.00 (0.91 – 1.22)	1.21 (0.39 – 4.11)	1.96 (0.41 – 6.15)

The mean GSS for all students was 7.6±5.4, with third-year students scoring the lowest (6.89) and fifth-year students scoring the highest (9.26) (Figure 2). In the unadjusted analysis, there was no discernible relationship between GSS and year of study.

Only anger, suicidal thoughts, and substance use remained strongly correlated with the study year after controlling for age and perceived COVID-19 risk. Fourth-year students showed significantly less anger than first-year students. Second-year students had considerably lower rates of suicidal ideation and substance use, while fourth-year students had significantly lower rates of both. Throughout the trial, all other domains remained consistent. Age and perceived COVID risk were considered, and neither PSS nor GSS substantially correlated with the student's year in PSAU.

Discussion

This study aimed to examine how the COVID-19 pandemic affected medical students' mental health symptoms. Anxiety and depression were the most frequent psychiatric symptoms mentioned by the medical students.

The students reported fewer instances of dissociation, psychosis, and substance usage. According to the COVID-19 Student Stress Questionnaire and the perceived stress scale, 8.3% of individuals had high-stress levels. The final-year students had the highest mean PSS, whereas the fourth-year students had the highest mean GSS. We contrasted our findings with research on student stress levels and psychiatric symptoms that have been published.

Medical students exhibited significantly higher rates of diagnosed mood disorders, diagnosed anxiety disorders, suicidal thoughts, and psychological distress than the overall population of postsecondary graduates. Natalia et al.[6] used the Fear of COVID-19 (FCV-19) Scale and the Depression, Anxiety, and Stress Scale-21 to assess FCV-19, anxiety, stress, and depression. Out of 1027 samples, 44.6% had stress, 47.8% had anxiety, and 18.6% had depression. The gender (P=0.000), educational stage (P=0.000), and the comorbidity factor (P=0.001 for stress and anxiety, P=0.036 for depression, and P=0.000 for FCV-19) had a significant association with stress, anxiety, depression, and FCV-19 in medical students.

According to several studies, stress, anxiety, and depression are more common among medical students. Suicidal thoughts, moderate to severe psychological distress, mood and anxiety disorders, and clinical training were all linked to being a medical student. Anxiety affects about one in three medical students worldwide, and it is especially common among those from the Middle East and Asia. A study by Maser et al.[10] showed that relative to the general population of postsecondary graduates aged 20–34, medical students aged 20–34 had significantly higher rates

of diagnosed mood disorders, anxiety disorders, suicidal ideation, and psychological distress. In a study by Harries et al.[11], most students (74.7%) agreed the pandemic had significantly disrupted their medical education. .

A study by Huckins et al.[12] included 217 undergraduate students, with 178(82.0%) students providing data during the Winter 2020 term. The authors assessed the differences in behaviors and self-reported mental health collected during the Winter 2020 term compared to previous terms in the same cohort were modeled using mixed linear models. Compared with prior academic terms, individuals in the Winter 2020 term were more passive, anxious, and depressed.

Another study performed by Liu et al.[13] on 217 medical students found that anxiety symptoms and depression were observed in 22.1% and 35.5% of students, respectively, during the pandemic. There were no significant differences in students, in terms of gender and grade, for the prevalence of depression and anxiety. COVID-19 has exacerbated uncertainty about one's personal and family relationships. This raises the stress level of medical students, combined with the uncertainties surrounding their education and skill development during medical school. In a study by Iqbal et al.[14], more than half of all respondents (n=353) were affected by depression (51.3%), anxiety (66.9%) and stress (53%). Morbidity was found to be higher in 5th-semester students rather than students in 2nd-semester students. Females reported higher scores as compared to their male counterparts.

More than half of the medical students in KSA indicated signs of mania, depression, and anxiety. According to the perceived stress scale and the COVID-19 Students Stress Questionnaire, 88.1% and 57.8% of students indicated moderate and high levels of stress, respectively. A thorough analysis of the causes of this high level of stress and other psychiatric symptoms is necessary. In order to prioritize the students for future management, this analysis should be supplemented by routine assessments of the mental health of medical students. Moreover, our findings are consistent with other research on the baseline frequency of depression and anxiety among Saudi medical students. Ewid et al.[15] conducted the study to assess the prevalence of depression, stress, and anxiety symptoms among Sulaiman Al Rajhi University (SRU) 278 medical students during the quarantine and while learning online shortly after the announcement of documented COVID-19 cases in KSA. Depression, anxiety, and stress symptoms were found in 23%, 11%, and 6% of students, respectively. Females were more likely to have anxiety (P=0.03) than males. The Fear of COVID-19 Scale was positively correlated with all depression, anxiety, and stress components (depression: r=0.36, anxiety: r=0.45, and stress: r=0.39, P<0.001 for all cases). The authors highlighted the importance of mental health screening for female students, students of low socioeconomic status, and relatives of COVID-19 cases.

Strong recommendations on medical students' ongoing education growth during crises and potential pandemics are required. We would also include recommendations for how medical schools could assist mental health. Future studies should examine both potential benefits and negatives, such as whether medical schools have kept up with the rise in understanding of mental health and investigations of attitudes toward stigmatization of mental health.

Conclusion

According to the study's findings, graduate medical students bear an immense load in terms of their mental health. They need proper care as soon as possible because this is likely to have a long-term negative impact on their performance. Therefore, having a clinical psychologist available to conduct regular evaluations of medical students is essential. Depending on the resources available and the needs identified by the ministry, these psychologists may be hired at the institutional or ministry level.

Competing Interests

The authors declare that they have no competing interests.

Acknowledgments

This publication was supported by the Deanship of Scientific Research at Prince Sattam bin Abdulaziz University, Al-Kharj, Saudi Arabia. In addition, we thank those who participated and contributed to the study.

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International Journal of Biomedicine 13(4) (2023) 341-344
http://dx.doi.org/10.21103/Article13(4)_OA18

ORIGINAL ARTICLE

Microbiology

INTERNATIONAL JOURNAL OF BIOMEDICINE

The Antibiotic Resistance Genes *bla*$_{SHV}$, *bla*$_{OXA-48}$, *bla*$_{TEM}$ and *bla*$_{IMP}$ in *Pseudomonas aeruginosa* Isolated from Respiratory Tract Infections in Baghdad, Iraq

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Abstract

***Background*:** *Pseudomonas aeruginosa* (*P. aeruginosa*) is the most common pathogen associated with respiratory tract infections. Our study aimed to detect the antibiotic resistance profile and some antibiotic resistance genes of local isolates of P. aeruginosa from respiratory tract infections and to determine the biofilm formation by these isolates.

***Methods and Results*:** Two hundred sputum samples were obtained from patients with CF from different hospitals in Baghdad from November 2022 to February 2023. Biochemical tests and the VITEK-2 system were used to identify P. aeruginosa isolates. The disc diffusion technique was used in the antibiotic susceptibility test, and the results were identified according to CLSI guidelines 2020. Biofilm formation was performed by the microtiter-plate method and determined using an ELISA reader at OD570. The PCR was performed to detect the *bla*$_{SHV}$ gene, *bla*$_{TEM}$ gene, *bla*$_{IMP}$ gene, and *bla*$_{OXA-48}$ gene.

Sixty (30%) isolates of P. aeruginosa were identified from 200 sputum samples.The results showed that 93.4% of the isolates were resistant to Amoxicillin-Clavulanic acid, 90% to Nitrofurantoin and Cefepime, 88.4% to Cefotaxime, 85% to Doxycycline, 83.4% to Tobramycin, 81.7% to Tetracycline and 80% to Meropenem. In comparison, 91.6% were sensitive to Ofloxacin, 68.3% to Azithromycin, and 36.6% to Chloramphenicol. All P. aeruginosa isolates were identified as MDR. The results revealed that 55% of the isolates produced strong biofilms, 38.3% produced moderate biofilms, and 6.7% produced weak biofilms. The rates of *bla*$_{SHV}$, *bla*$_{TEM}$, *bla*$_{IMP}$, and *bla*$_{OXA-48}$ genes were 28.3%, 60%, 26.6%, and 68.3%, respectively.

***Conclusion*:** This study revealed that all isolates showed MDR phenotype. Biofilm formation by P. aeruginosa isolates and the variation in the incidence of antibiotic resistance encoding genes, in addition to the abuse and overuse of antibiotics, are significant reasons for the progress and spread of antibiotic resistance.**(International Journal of Biomedicine. 2023;13(4):341-344.)**

Keywords: *P. aeruginosa* • cystic fibrosis • antibiotic resistance • biofilm

For citation: Owaif HAA, Aldulaimy MK, Abdulateef SA. The Antibiotic Resistance Genes *bla*$_{SHV}$, *bla*$_{OXA-48}$, *bla*$_{TEM}$ and *bla*$_{IMP}$ in *Pseudomonas aeruginosa* Isolated from Respiratory Tract Infections in Baghdad, Iraq. International Journal of Biomedicine. 2023;13(4):341-344. doi:10.21103/Article13(4)_OA18

Abbreviations

CF, cystic fibrosis; **AR**, antibiotic resistance; **MDR**, multidrug resistant; **PCR**, polymerase chain reaction

Introduction

The opportunistic *Pseudomonas aeruginosa* (*P. aeruginosa)* is a common bacterium that can survive in various environments but prefers humid conditions. It is a common bacterium found in hospitals that causes nosocomial severe infections, specifically in severely sick and immuno-compromised patients. *P. aeruginosa* is implicated in various diseases, such as respiratory and urinary tract infections, infection of individuals with thermal burns, and wound and soft tissue infections.[(1)] *P. aeruginosa* is a prevalent infection in the lungs of patients suffering from cystic fibrosis (CF), and it is linked to frequent pulmonary exacerbations as well as significant mortality and morbidity. This bacterium can live in the lungs of CF patients for years.[(2)] Many of the antimicrobial medications that are currently used for treating severe

infections caused by multidrug-resistant (MDR) strains, such as carbapenems, fluoroquinolones, and third generation cephalosporins, have been found to be ineffective against *P. aeruginosa* strains.[3,4] The treatment of *P. aeruginosa* infections can become progressively challenging as *P. aeruginosa* antibiotic resistance (AR) increases. *P. aeruginosa* possesses many resistance mechanisms that contribute to eradication failure and persistent infections, including porin loss and efflux pump overexpression, as well as the synthesis of inactivating enzymes such as ß-lactamases.[5,6] Clinically approved antibacterial agents may be inefficient in treating infections by *P. aeruginosa* due to the bacterium's capacity to produce biofilms. The production of biofilms allows *P. aeruginosa* to survive externally in hostile conditions and improves colonization inside the host.[7] Additionally, biofilms serve as diffusion barriers, preventing antibiotics from entering bacterial cells.[8,9]

Our study aimed to detect the AR profile and some AR genes of local isolates of *P. aeruginosa* from respiratory tract infections and to determine the biofilm formation by these isolates.

Materials and Methods

Two hundred sputum samples were obtained from patients with CF from different hospitals in Baghdad from November 2022 to February 2023. They were plated on McConkey and cetrimide agar for 24 hours at 37°C. Biochemical tests and the VITEK-2 system were used to identify *P. aeruginosa* isolates. The disc diffusion technique was used in the antibiotic susceptibility test, and the results were identified according to CLSI guidelines 2020. The antibiotics used were Azithromycin (AZM) 30 µg, Doxycycline (DOX) 30 µg, Chloramphenicol (CHL) 30 µg, Tetracycline (TE) 30 µg, Nitrofurantoin (NF) 100 µg, Amoxicillin-Clavulanic acid (AMC) 20/10 µg, Ofloxacin (OF) 5 µg, Norfloxacin (NOR) 10 µg, Meropenem (MEM) 10 µg, Ticarcillin (TC) 75 µg, Cefepime (FEP) 30 µg, Tobramycin (TOB) 10 µg, Cefotaxime (CTX) 30 µg and Imipenem (IMP) 10 µg. Biofilm formation was performed by the microtiter-plate method and determined using an ELISA reader at OD570. The PCR was performed to detect the bla_{SHV} gene, bla_{TEM} gene, bla_{IMP} gene, and bla_{OXA-48} gene (Table 1).

Table 1.

Primer sequence.

Primer	Sequence: 5' → 3'	Amplicon size (bp)	Ref.
bla_{SHV}	F: TCAGCGAAAAACACCTTG R: TCCCGCAGATAAATCACC	472	(10)
bla_{TEM}	F: TTTCGTGTCGCCCTTATTCC R:ATCGTTGTCAGAAGTAAGTT	403	(11)
bla_{IMP}	F:ACCGCAGCAGAGTCTTTGCC R:ACAACAAGTTTTGCCTTACC	587	(12)
bla_{OXA-48}	F:GCGTGGTTAAGGATGAACAC R:CATCAAGTTCAACCCAACCG	438	(13)

Ref -Reference

Results

Sixty (30%) isolates of P. aeruginosa were identified from 200 sputum samples. The pattern of distribution of *P. aeruginosa* isolates based on gender and age groups is presented in Figure 1. The results showed that 93.4% of the isolates were resistant to Amoxicillin-Clavulanic acid (AMC), 90% to Nitrofurantoin (NF) and Cefepime (FEP), 88.4% to Cefotaxime (CTX), 85% to Doxycycline (DOX), 83.4% to Tobramycin (TOB), 81.7% to Tetracycline and 80% to Meropenem. In comparison, 91.6% were sensitive to Ofloxacin (OF), 68.3% to Azithromycin (AZM), and 36.6% to Chloramphenicol (CHL) (Table 2). All *P. aeruginosa* isolates were identified as MDR. The results revealed that 55% of the isolates produced strong biofilms, 38.3% produced moderate biofilms, and 6.7% produced weak biofilms. The rates of bla_{SHV}, bla_{TEM}, bla_{IMP}, and bla_{OXA-48} genes were 28.3%, 60%, 26.6%, and 68.3%, respectively (Table 3). Table 4 indicates the relation between biofilm formation and antibiotic resistance.

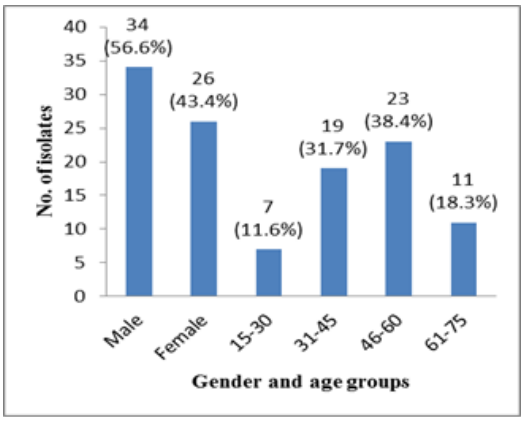

Fig. 1. *Distribution of P. aeruginosa isolates.*

Table 2.

Antimicrobial resistance of P. aeruginosa isolates.

Class	Antibiotic	Sensitive n (%)	Resistance n (%)
Macrolides	AZM	41 (68.3)	19 (31.7)
Tetracyclines	DOX	9 (15)	51 (85)
	TE	11 (18.3)	49 (81.7)
Beta-lactam and Beta-Lactamase Inhibitor	AMC	4 (6.6)	56 (93.4)
	TC	14 (23.3)	46 (76.7)
Nitrofurantoin	NF	6 (10)	54 (90)
Fluroquinolones	OF	55 (91.6)	5 (8.4)
	NOR	17(28.4)	43 (71.6)
Carbapenems	MEM	12 (20)	48 (80)
	IMP	17 (28.4)	43 (71.6)
Cephalosporin	FEB	6 (10)	54 (90)
	CTX	7 (11.6)	53 (88.4)
Chloramphenicol	CHL	22 (36.6)	38 (63.4)
Aminoglycosides	TOB	10 (16.6)	50 (83.4)

Table 3.
The antibiotic resistance genes among P. aeruginosa isolates.

Genes	No. of isolates	%
bla_{SHV}	17	28.3
bla_{TEM}	36	60
bla_{IMP}	16	26.6
bla_{OXA-48}	41	68.3
bla_{TEM}, bla_{OXA-48}	36	60
bla_{TEM}, bla_{OXA-48}, bla_{SHV}, bla_{IMP}	11	18.3

Table 4.
The relation between antibiotic resistance and biofilm formation in P. aeruginosa isolates.

Antibiotic	Strong (n=33)	Moderate (n=23)	Weak (n=4)
	n (%)	n (%)	n (%)
AZM	11 (33.3)	7 (30.4)	1 (25)
DOX	29 (87.8)	20 (86.9)	2 (50)
TE	27 (81.8)	18 (78.2)	4 (100)
AMC	31 (93.9)	21 (91.3)	4 (100)
TC	23 (69.7)	20 (86.9)	3 (75)
NF	29 (87.8)	21 (91.3)	4 (100)
OF	3 (9)	2 (8.6)	0 (0)
NOR	23 (69.7)	18 (78.2)	2 (50)
MEM	26 (78.7)	19 (82.6)	3 (75)
IMP	24 (72.7)	16 (69.5)	3 (75)
FEB	29 (87.8)	21 (91.3)	4 (100)
CTX	31 (93.9)	18 (78.2)	4 (100)
CHL	22 (66.6)	13 (56.5)	3 (75)
TOB	27 (81.8)	20 (86.9)	3 (75)

Discussion

P. aeruginosa is the most common pathogen in the respiratory tract of patients who suffer from CF and other chronic infections. The low antibiotic sensitivity is one of the most concerning aspects of this bacterium.[14] In the current study, 60(30%) of 200 sputum samples tested positive for *P. aeruginosa*, whereas 70% were positive for other bacterial isolates. This agreed with a study by Wang et al., who reported that *P. aeruginosa* was the pathogen with the most significant sputum isolation rate (23.8%).[15] Distribution of *P. aeruginosa* isolates among males was 56.6%, compared to females (43.4%); males were more susceptible to bacterial infection than females due to higher exposure to numerous unfavorable environmental variables.[16] The age group 46-60 years had the highest percentage of the AR isolates (38.4%). The isolates were highly susceptible to Ofloxacin (91.6%) and highly resistant to Amoxicillin-Clavulanic acid (93.4%). This result was close to that of a study by Motbainor et al., who showed that 100% of *P. aeruginosa* isolates were Amoxicillin-Clavulanic acid resistant.[17] All isolates were MDR, and this result agreed with Abbas et al., who reported that 100% of *P. aeruginosa* isolates were MDR.[18] AR may be linked to alterations in bacterial enzymes as well as patients' overuse and abuse of antibiotics.[19,20] The bla_{SHV} gene was detected in 28.3% of the isolates, bla_{TEM} in 60%, bla_{IMP} in 26.6%, and bla_{OXA-48} in 68.3% of the isolates. At the same time, other studies show 6.6% for bla_{SHV},[21] 100% for bla_{TEM},[22] 42.8% for bla_{IMP},[23] and 36.1% for bla_{OXA-48}.[24] The incidence of resistance genes varies greatly between studies, which may lead to variation in infection management guidance. The current study showed a correlation between biofilm formation and resistance to antibiotics. These findings were consistent with previous studies, which indicated that bacteria in planktonic form are less resistant to antibiotics than bacteria in biofilm form.[25] Horizontal gene exchange, on the other hand, is considerably increased in biofilms because resistant bacteria can transfer resistance genes to other bacteria.[26,27]

In conclusion, this study revealed that all isolates showed MDR phenotype. Biofilm formation by *P. aeruginosa* isolates and the variation in the incidence of AR encoding genes, in addition to the abuse and overuse of antibiotics, are significant reasons for the progress and spread of AR. The elevated prevalence of MDR *P. aeruginosa* emphasizes the critical importance of establishing alternate treatment methods.

Competing Interests

The authors declare that they have no competing interests.

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International Journal of Biomedicine 13(4) (2023) 345-349
http://dx.doi.org/10.21103/Article13(4)_OA19

ORIGINAL ARTICLE

Antimicrobial Agents

INTERNATIONAL JOURNAL OF BIOMEDICINE

In Vitro Comparisons of Minimal Inhibitory Concentrations between NaOCL, CHX, MTAD and EDTA against *Candida Albicans*

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Abstract

The aim of this in vitro study was to evaluate the minimal inhibitory concentration (MIC) of 3% NaOCl, 2% CHX, MTAD, and EDTA against *Candida albicans (C. albicans)*.

Methods and Results: Certified strain of *C. albicans* (ATCC 10231 OXOID, Hampshire, UK) was used to determine the MIC of 3% NaOCl, 2% CHX, MTAD, and EDTA in vitro. The broth dilution method was used to determine the MIC and to ensure the test was highly accurate. *C. albicans* and respective irrigants were gradually placed into the appropriate test tubes, starting from 1 mL to 0.06 mL of irrigant (getting halved each time). The test tubes were incubated at 37°C for 24 h. MIC was then recorded as the lowest concentration of irrigant that inhibited microbial growth, based on based on spectrophotometry. Our study showed that some of the tested irrigants retained an antifungal effect after dilution, which is valuable because dilution reduces toxicity. 3% NaOCl has an efficient antifungal effect against *C. albicans* both at full concentration and when diluted fivefold. The antifungal effect of 2% CHX for *C. albicans* cultures increases with its dilution. MTAD retains a good antifungal effect even when diluted fivefold.**(International Journal of Biomedicine. 2023;13(4):345-349.)**

Keywords: *Candida albicans* • minimal inhibitory concentration • MTAD • CHX • NaOCl • EDTA

For citation: Shabani DB, Teneqja AD. In Vitro Comparisons of Minimal Inhibitory Concentrations between NaOCL, CHX, MTAD and EDTA against *Candida Albicans*. International Journal of Biomedicine. 2023;13(4):345-349. doi:10.21103/Article13(4)_OA19

Introduction

Microorganisms can be a major cause of pulp and periapical pathosis.[1-3] To avoid the formation of periapical lesions, endodontic techniques such as cleaning, shaping root canals, and sealing the entire root canal are preventive measures for fluids entering into the root canal that serve as nutrients for the remaining microbes within the canals.[4,5]

Candida albicans (C. albicans) is an opportunistic pathogen but also a part of the human microbiota and is present in different areas, such as the oral cavity, skin, gastrointestinal tract, and vagina.[6] The root canal system is frequently subjected to microorganisms' colonization, spreading into infections and then persistent inflammation. Different studies have found numerous species to be responsible for causing apical periodontitis, but highlighted *C. albicans* as the most frequently isolated one.[7] In vulnerable individuals, when conditions are favorable, *C. albicans* prevalently behaves as a pathogen, causing oral candidal infections and complications with any other endodontic treatments.[8] *C. albicans* plays an important role in endodontic treatment failure; therefore, prompt intervention is important to eradicate the infection. Not many remedies for oral diseases caused by *C. albicans* have been discovered; hence, it is necessary to find new compounds.[9]

As established, fungi are the cause of failures of root canal treatment and are more common in secondary endodontic infections.[10] One-third of people have fungi as their normal flora, but sometimes *C. albicans* is one of the most frequent causes of oral infections.[11] Approximately 30%-45% of healthy adults carry this fungus; meanwhile, in patients with human immunodeficiency virus, this rate is at 95%, meaning

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that in patients with an impaired immune system, this species can expand.[12-14]

To date, NaOCl, EDTA, CHX, and MTAD are available as irrigant solutions for endodontic use, but each one presents advantages and disadvantages. Sodium hypochlorite (NaOCl) is an efficient disinfection organic solvent, commonly used by dentists for cleaning root canals, that causes dentinal degeneration through collagen dissolution; however, it cannot remove the smear layer.[15] Chlorhexidine (CHX) exhibits antimicrobial activity and biocompatibility but has no tissue-dissolving capabilities.[16] MTAD combines antibiotics (doxycycline), chelator (citric acid), and detergent (Tween-80). The citric acid chelator contributes to smear layer removal, allowing doxycycline to penetrate dentinal tubules with opened orifices due to the detergent effect.[17] Ethylenediaminetetraacetic acid (EDTA) is a neutral or slightly alkaline solution used as an irrigant. It is prevalently used for smear layer removal.[18] Although little is known about its antibacterial efficacy, some authors have described its strong effect on removing *C. albicans* in root canals.[19]

The aim of this in vitro study was to evaluate the minimal inhibitory concentration (MIC) of 3% NaOCl, 2% CHX, MTAD, and EDTA against *C. albicans.*

Materials and Methods

Candida strain

In this study, *C. albicans* (ATCC 10231 OXOID, Hampshire, UK) was used to determine the antimicrobial effect of irrigants *in vitro*. *C. albicans* was grown in a culture medium prepared according to the manufacturer›s guidelines. The strain suspension was transferred into Sabouraud dextrose and incubated overnight at 30°C. The colonies were then isolated and maintained by weekly reinoculations in the same media.

Standardization of microorganisms

Brain heart infusion broth (BHI-Oxoid LTD., Hampshire, UK) was inoculated with *C. albicans* and incubated for 6–7 h at 37°C to achieve a mean optical density of 0.5 McFarland (approximately equal to 1.5×10^8 CFU/mL). Then, 1 mL aliquots of each suspension culture were transferred to the required number of sterile tubes. All procedures were performed using sterilized instruments and reagents.

Selection of irrigants

The following irrigants were used:

- 3% NaOCl (ChlorcID, Ultradent Products, Inc. South Jordan, UT, USA)
- 2% CHX (Consepsis, Ultradent Products, Inc. South Jordan, UT, USA)
- MTAD (Dentsply Tulsa Dental, Tulsa, OK, USA)
- 17% EDTA (CALASEPT EDTA, Nordiska Dental, Ängelholm, Sweden)

Determination of the minimal inhibitory concentration

MIC value represented the lowest concentration that inhibited 100% of the fungal growth and was determined by the minimum concentration of a respective irrigant. Therefore, the MIC was used as a reference criterion for the susceptibility of the microorganisms to the compound. The broth dilution method was used to determine the MIC and to ensure the test was highly accurate. C. albicans and respective irrigants were gradually placed into the appropriate test tubes, starting from 1 mL to 0.06 mL of irrigant (getting halved each time). The test tubes were incubated at 37°C for 24 h. MIC was then recorded as the lowest concentration of irrigant that inhibited microbial growth, based on spectrometry at the wavelength of 540 nm (Smart-CCD Spectrophotometer).

Statistical analysis was performed using *statistical software* package *SPSS version 20.0* (*SPSS* Inc, Armonk, NY: IBM Corp).

Results

Table 1 shows MICs and standard deviations (SD) of tested materials according to absorbance rate. The higher the value of the absorbance rate, the smaller the antimicrobial effectiveness of the tested substance. By diluting the substances, their antimicrobial activity was reduced.

The higher the value of the purity percentage, the greater the antimicrobial effect (Table 2). By diluting the substances, their antifungal activity was reduced. The antifungal effect of 3% NaOCl decreased with its increasing dilution: the absorbance increased from 0.16±0.01 (at 1mL dilution) to 0.58±0.005 (at 0.06 mL dilution); similarly, purity decreased from 70.1±1.223 (at 1mL dilution) to 26.45±3.13 (at 0.06 mL dilution) (Figure 1).

Table 1.

MICs of tested materials according to the absorbance rate.

Irrigants	1000 uL		500 uL		250 uL		125 uL		60 uL	
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
3% NaOCl	0.16	0.01	0.17	0.01	0.39	0.02	0.61	0.06	0.58	0.05
2% CHX	0.62	0.01	0.63	0.01	0.65	0.15	0.18	0.001	0.04	0.003
MTAD	0.04	0.001	0.08	0.01	0.31	0.01	0.32	0.003	0.35	0.001
17% EDTA	0.19	0.01	0.21	0.01	0.49	0.01	0.56	0.01	0.62	0.01

Table 2.

MICs of tested materials according to the purity percentage.

Irrigants	1000 uL		500 uL		250 uL		125 uL		60 uL	
	x̄ [%]	SD	x̄ [%]	SD	x̄ [%]	SD	x̄ [%]	SD	x̄ [%]	SD
3% NaOCl	70.11	1.22	63.44	2.04	41.53	1.62	24.63	3.23	26.45	3.13
2% CHX	23.68	0.86	23.32	0.77	22.95	8.35	66.89	1.59	93.33	5.44
MTAD	99.18	0.00	82.67	0.57	48.74	1.16	48.24	0.46	44.52	0.03
17% EDTA	63.87	1.75	62.46	0.12	32.19	0.60	27.81	1.04	24.21	1.00

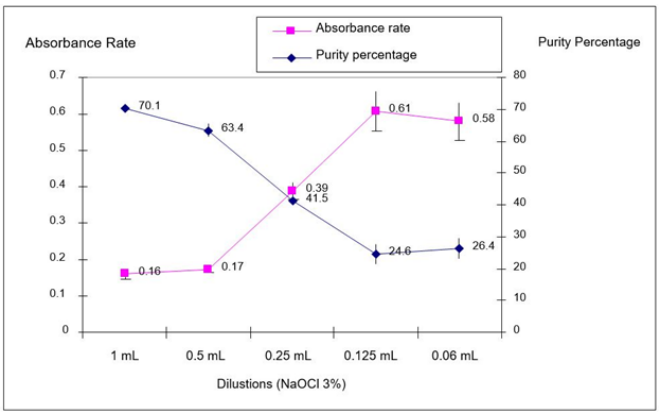

Fig. 1. *MIC of 3% NaOCl against C. albicans.*

The antifungal effect of 2% CHX for *C. albicans* cultures increased with its dilution: the absorbance decreased from 0.62±0.01 (at 1mL dilution) to 0.04±0.003 (at 0.06 mL dilution); similarly, purity increased from 23.7±0.86 (at 1mL dilution) to 93.3±5.44 (at 0.06 mL dilution) (Figure 2).

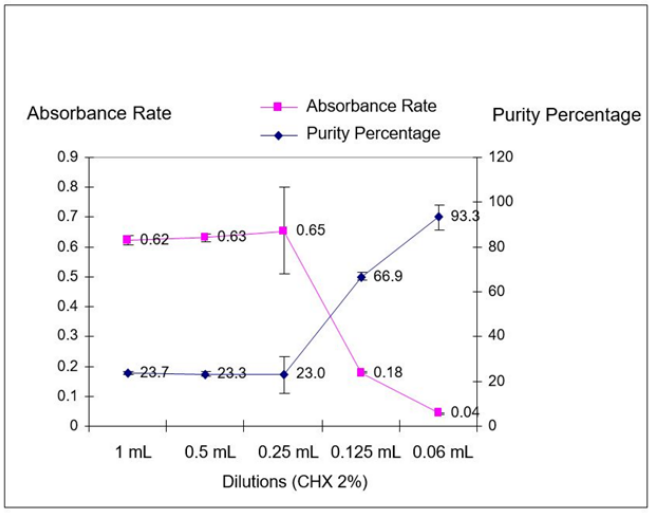

Fig. 2. *MIC of 2% CHX against C. albicans.*

The antifungal effect of MTAD exhibited variable outcomes, with a general tendency to decrease with increasing dilution: absorbance increased from 0.04±0.001 (at 1mL dilution) to 0.35±0.001 (at 0.06 mL dilution); similarly, purity decreased from 99.2±0.0 (at 1mL dilution) to 44.5±0.03 (at 0.06 mL dilution) (Figure 3). Notably, the antifungal effect of 17% EDTA decreased as it was diluted: absorbance increased from 0.19±0.01 (at 1mL dilution) to 0.62±0.001(at 0.06 mL dilution), while purity decreased from 63.9±1.75 (at 1mL dilution) to 24.21±1.0 (at 0.06 mL dilution) (Figure 4).

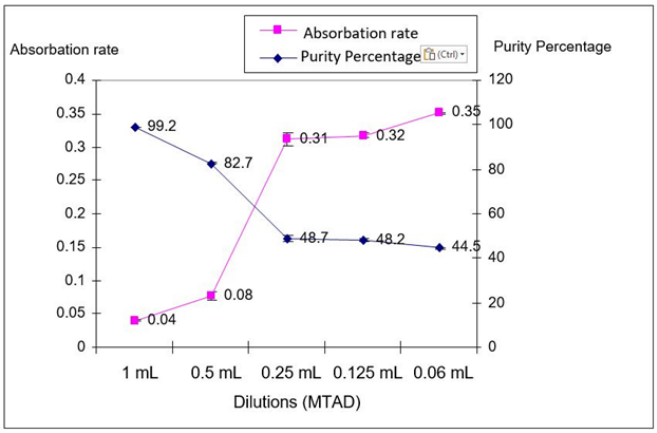

Fig. 3. *MIC of MTAD against C. albicans.*

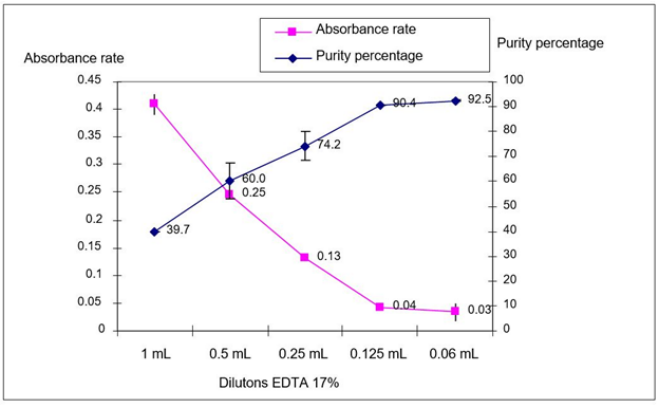

Fig. 4. *MIC of 17% EDTA against C. albicans.*

Discussion

The aim of this in vitro study was to evaluate the minimal inhibitory concentration (MIC) of 3% NaOCl, 2% CHX,

MTAD, and EDTA against *C. albicans. C. albicans* was selected because it is the most dominant fungal species in persistent endodontic infections. ATCC 10212 strain is a commonly used quality control for *in vitro* antifungal studies.[20]

Our study showed that some of the tested irrigants retained an antifungal effect after dilution, which is valuable because dilution reduces toxicity. Notably, a fivefold dilution of 3% NaOCl caused purity to decrease from 70.11% to 26.45%. Antifungal activity for NaOCl remained with 41% for 0.25 mL dilution. Regarding the efficacy of 3% NaOCl against *Candida*, it has been shown that its dilution reduces the antifungal effect. Until dilution of 3% NaOCl three times, the irrigant had a good antifungal effect against *C. albicans*, which meant that the MIC of NaOCl against *C. albicans* was 0.7%.

Antifungal activity for CHX increased from 23% at 1000 uL to 93% after dilution at 60 uL. Similarly, diluting CHX increased its antifungal effect. Meanwhile, the antifungal effect of MTAD was decreased from 99% to 44% after being diluted five-times. This result still showed that MTAD had quite a good effect even after diluting it five times. EDTA 17% showed lower antifungal activity after being diluted four times, from 69% to 24%. EDTA is a substance with a very good chelating effect, is biocompatible with periapical tissue, and has an antifungal effect against *C. albicans*.[21] In other studies, in which an agar diffusion method was used, EDTA 17% has shown the best antifungal effect compared to other antifungal drugs and test solutions.[22]

NaOCl is a disinfecting and oxidizing agent with bactericidal, virucidal, and fungicidal activity. Due to its antimicrobial and tissue-dissolving properties, it is commonly used by dentists for cleaning root canals.[15] Although it has a positive antimicrobial effect, the quantity could provoke NaOCl extrusion with the consequent bleeding or other severe symptoms in the patients.[23] Nevertheless, there is a lack of agreement about the exact concentration of NaOCl to use. Moreover, its efficacy is strongly associated with the volume and frequency of irrigation.[24] In relation to these factors, NaOCl is essential for the effective shaping and cleaning of root canals, for its proteolytic and dissolution capacity, and for its debridement properties. Hence, it remains a common compound on which studies regarding its exact concentration were necessary.[25]

Meanwhile, CHX is a disinfectant and antiseptic compound used for multiple reasons, including dental use. CHX acts as an antibacterial thanks to its chemical structure, for which its positively charged lipophilic/hydrophobic form interacts with phospholipids and lipopolysaccharides of the bacterial cell membrane and consequently alters their osmotic equilibrium.[16] Regarding its antifungal activity, numerous studies help to assess and encourage its use, especially against *C. albicans*. Moreover, some studies have also highlighted its use as a substitute for NaOCl for its lower cytotoxicity and its efficient clinical performance.[26]

MTAD is an aqueous solution constituted of 3% antibiotics doxycycline, 4.25% of the demineralizing agent citric acid, and another small percentage of TWEEN 80 detergent. Its composition makes possible its solubilization in water.[17] MTAD is commonly used as an endodontic irrigant with antibacterial activity and for its ability to remove the smear layer. Not many studies have been made about its antifungal activity, but it is frequently used in combination with NaOCl to increase its efficacy.[27] As for EDTA, although little is known about its antibacterial efficacy, some authors have described its strong effect on the removal of *C. albicans* in root canals.[19] EDTA acts by reducing colonization and growth of *C. albicans*, interfering with its morphogenesis and adherence capacity.[22]

Based on the best antifungal effect, the test substances are sorted according to the size of the inhibition zone: 3% NaOCl > 2% CHX > MTAD > 17% EDTA. Our data are consistent with those of other authors who have evaluated the antimicrobial effect of CHX and have compared it to the effect of NaOCl. CHX 2% gel and solution and NaOCl 0.5%-5.25% have eliminated *C. albicans*.[28,29] Despite that, our study had limitations. The main limitation of this *in vitro* study was that the findings might not be representative of *in vivo* outcomes; therefore, additional *in vitro* and *in vivo* studies are needed to confirm the apparent antibacterial activities and MICs of these irrigants against *C. albicans* to support their clinical applications.

In conclusion, the results of this study indicate that 3% NaOCl has an efficient antifungal effect against *C. albicans* both at full concentration and when diluted fivefold. The antifungal effect of 2% CHX for *C. albicans* cultures increases with its dilution. MTAD retains a good antifungal effect even when diluted fivefold.

Competing Interests

The authors declare that they have no competing interests.

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International Journal of Biomedicine 13(4) (2023) 350-355
http://dx.doi.org/10.21103/Article13(4)_OA20

ORIGINAL ARTICLE

Experimental Biology

INTERNATIONAL JOURNAL OF BIOMEDICINE

Erythropoietin Protective Role Against Methotrexate Testicular Adverse Effects

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Abstract

Background: The adverse effects of cytotoxic medications like methotrexate, particularly reproductive damage reported in the numerous experimental studies, limit their effectiveness as anticancer therapies. The current study's objectives were to identify potential histological and immune-histochemical unfavorable changes in the testicles due to methotrexate medication and to assess any possible protective effects of erythropoietin (EPO).

Methods and Results: The study included 60 mature male albino rats weighing 200-250 g. The animals were divided equally into three groups, each with 20 rats. In Group 1, the control group, the animals received intraperitoneal injections of normal saline twice a week for nine weeks at a dose of 0.5 mg/kg. For nine weeks, animals in Group 2 received intraperitoneal injections of methotrexate hydrate at 0.5 mg/kg twice a week. Animals in Group 3 received subcutaneous injections of 100 IU/kg recombinant human EPO once a week for nine weeks and intraperitoneal injections of methotrexate hydrate at a dosage of 0.5 mg/kg twice a week for nine weeks. Each rat was weighed every week throughout the examinations. An ELISA technique was used to measure the levels of testosterone, malondialdehyde, total antioxidant capacity, and ROS in blood serum. Morphological and histopathological changes in testicular tissue were assessed. The body weight in the rats treated with methotrexate was considerably lower than in control group rats. EPO showed clear androgenic and antioxidant activities and reduced the adverse effects of methotrexate on testicular histology. Our results suggest further research into the use of EPO as a drug to protect patients from the adverse effects of methotrexate.**(International Journal of Biomedicine. 2023;13(4):350-355.)**

Keywords: testicular tissue • erythropoietin • methotrexate • oxidative stress

For citation: Ali AHA, Alotaibi TR, Arab THA, Zaila AH, Alharbi BM, Alharbi ZS, Alduhaymi AM, Alamer AF, Alquraini FA, Alzmami NK, Alharbi TA, Alkhurayyif AO, Alotaibi NON, Alanazi AMM, Alasiri AMA. Erythropoietin Protective Role Against Methotrexate Testicular Adverse Effects. International Journal of Biomedicine. 2023;13(4):350-355. doi:10.21103/Article13(4)_OA20

Abbreviations

EPO, erythropoietin; **MDA**, malondialdehyde; **ROS**, reactive oxygen species; **TAC**, total antioxidant capacity.

Introduction

One of the most dangerous side effects of chemotherapy is testicular destruction, which is commonly accompanied with oligozoospermia and azoospermia.(1) The antineoplastic medication methotrexate is frequently used to treat neoplasms. Many significant organs are affected by the side effects that patients who are receiving methotrexate medication experience. Numerous neoplastic diseases, such as acute lymphoblastic leukemia, non-Hodgkin's lymphoma, breast cancer, and testicular cancers, are treated with methotrexate.(2) Since methotrexate is an anti-metabolite medication, it is frequently

used to treat a variety of illnesses, including psoriasis and rheumatoid arthritis.[3] However, a significant danger is the unfavorable impact of methotrexate on rapidly growing organs. One of the methotrexate side effects is testicular damage. Oxidative stress, apoptotic alterations, inflammation, and changes in blood flow all play a role in how this effect manifests. [4] Testis and germ cell structure are harmed by oxidative stress. As a result, substances with antioxidant qualities may help shield testicular tissue from the adverse effects of oxidative stress from methotrexate. Erythropoietin (EPO), a glycoprotein hormone produced in the kidneys and secreted into the blood circulatory system, regulates erythropoiesis. EPO acts by binding to its cognate receptor (EPOR), which is expressed on the surface of erythroid progenitor cells. Signaling definitive erythrocyte progenitors via the EPOR leads to rescue from apoptosis, cell proliferation, expression of erythroid-specific proteins such as hemoglobin, and ultimately terminal differentiation into mature, enucleated definitive erythrocyte.[5] EPO is induced by hypoxia via the hypoxia-inducible factor family of transcription factors. Recently, it has been shown that EPO possesses cytoprotective properties in addition to its typical hematopoietic function.[6] As a growth factor, EPO is essential for cell development and neovascularization.

Our team previously investigated and evaluated the preventive effects of EPO and sildenafil intraperitoneally injected into adult rats who underwent testicular torsion and detorsion. The research showed that EPO treatment had milder histological changes than the control group, with sildenafil likely having an improved action.[7] Numerous studies have described the cytoprotective properties of EPO in various organs, including the brain, kidney, heart, and retina, and have suggested that EPO may have therapeutic value. However, little evidence is presented regarding the testicles. [8] The current study's objectives were to identify potential histological and immune-histochemical unfavorable changes in the testicles due to methotrexate medication and to assess any possible protective effects of EPO.

Materials and Methods

Our work was conducted in accordance with the PSA University's Al-Kharj Ethical Committee's guidelines for the use and care of animals in research (SCBR-136-2022). The protocol for this study was also created in compliance with the ethical standards of the Laboratory of the International Committees for the Protection of Animal Rights.

The study included 60 mature male albino rats weighing 200-250 g. Rats were kept in the vivarium at a temperature of 21–22°C with a 12-hour light/dark cycle, fed a conventional rat diet, and allowed unlimited access to water.

The experiments were performed in accordance with the norms for the humane treatment of animals, which are regulated by the International Guidelines of the Association for the Assessment and Accreditation of Laboratory Animal Care, following the protocol approved by the Institutional Animal Care and Use Committee of the PSAU (SCBR-136-2022).

We purchased methotrexate tablets (2.5 mg) from the Orion Corporation (Espoo, Finland). EPO (5000 IU) was purchased from Recormon (Roche Diagnostics GmbH, Mannheim, Germany).

Following two weeks of acclimation, the animals were divided equally into three groups, each with 20 rats. In Group 1, the control group, the animals received intraperitoneal injections of normal saline twice a week for nine weeks at a dose of 0.5 mg/kg. For nine weeks, animals in Group 2 received intraperitoneal injections of methotrexate hydrate at 0.5 mg/kg twice a week. Animals in Group 3 received subcutaneous injections of 100 IU/kg recombinant human EPO once a week for nine weeks and intraperitoneal injections of methotrexate hydrate at a dosage of 0.5 mg/kg twice a week for nine weeks.

Each rat was weighed every week throughout the examinations. Blood samples were drawn from the retro-orbital venous plexus using a capillary tube. The serum was separated by centrifugation and stored at -20°C. An ELISA technique was used to measure the levels of testosterone,[9] malondialdehyde (MDA), total antioxidant capacity (TAC), and ROS in blood serum. Then, ether inhalation anesthesia was given to the rats. The testicles were removed from the rats (Figure 1) at the designated dates via a median abdominal incision, stored, and then processed for paraffin slices. All specimens were kept in a 10% formol saline solution for three days to prepare them for paraffin sectioning. The testes were immediately removed and cured in 10% formal saline solution for three days. After a day of treatment with 70%, 90%, and finally absolute alcohol, the specimens were totally dehydrated and then subjected to three hours of treatment with ethyl alcohol at increasing concentrations. For a full day, the samples were benzene-cleared. The cleaned samples were submerged in paraffin wax three times for one hour each. After that, the samples were firmly embedded in paraffin wax. For general morphological analysis and the detection of histopathological changes, thin paraffin sections (3–4 microns) were prepared, mounted on charged glass slides, and stained with H&E. Mallory's trichrome stain was used to determine the different groups' tubular diameters and glycogen levels in the seminipherous tubules. In addition, the average number of seminiferous tubular germinal cells expressing caspase-3 in the study's various groups, as determined by sections stained with caspase-3, were studied.[10]

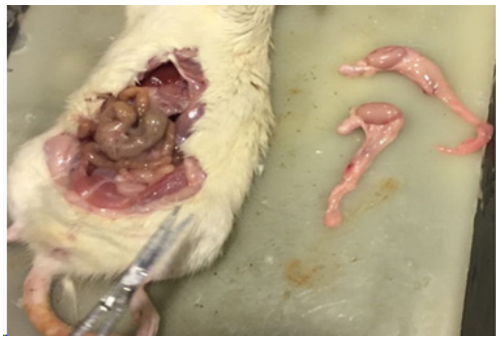

Fig. 1. *The removed testicles.*

Statistical analysis was performed using statistical software package SPSS version 17.0 (Chicago: SPSS Inc.). Baseline characteristics were summarized as frequencies and

percentages for categorical variables and as mean±SD for continuous variables. Multiple comparisons were performed with one-way ANOVA and Tukey HSD post-hoc test. A probability value of $P<0.05$ was considered statistically significant.

Results

The body weight in Group 2 was considerably lower than in Group 1 (Table 1). Compared to the control group, the TAC was not significantly altered by methotrexate treatment in Group 2. However, EPO administration in Group 3 raised antioxidant levels, compared to Group 1. Group 3 showed a significant increase in testosterone level and ROS compared to Group 2. Malondialdehyde levels in Group 2 did not differ from those of Group 1 (Table 2).

Table 1.

The body weight in the study groups.

Parameters	Group 1	Group 2	Group 3
Weight at the start of the experiment (g)	280 ± 4.33	231 ± 3.43	260 ± 5.22
Weight at the end of the experiment (g)	290 ± 3.11	210 ± 2.44	270 ± 5.31
Percentage (%)	+3.44 %	-9.09%	+ 3.7%

Table 2.

Effects of EPO and methotrexate treatment on the levels of testosterone, malondialdehyde, and ROS in serum.

	Group 1 (1)	Group 2 (2)	Group 3 (3)	Statistic
Testosterone, ng/ml	8.3±1.19	7.26±1.88	9.99±3.18	F=7.562, P= 0.0012 P_{1-2}=0.3139 P_{1-3}=0.0526 P_{2-3}=0.0009
TAC, mmol/g	5.77±2.99	5.11±2.23	8.77±3.98	F=7.674, P= 0.0011 P_{1-2}=0.7859 P_{1-3}=0.0106 P_{2-3}=0.0015
ROS, $\mu mol \cdot min^{-1}$	4.32±0.77	1.51±0.31	6.12±1.33	F=131.722, P= 0.0000 P_{1-2}=0.0000 P_{1-3}=0.0000 P_{2-3}=0.0000
MDA, nmol/l	2.39±0.56	2.11±0.44	2.94±1.22	F=5.361, P= 0.0074 P_{1-2}=0.5268 P_{1-3}=0.0923 P_{2-3}=0.0059

Sections from the control group displayed normal seminiferous tubule histology, an entire luminal spermatogenic series, and interstitial Leydig cells. Spermatogenic cells in various developmental stages and mature sperms were found in the lumen of the seminiferous tubules. Two cell types were present in seminiferous tubules: spermatogenic and Sertoli cells (Figure 2). Spermatogonia, spermatocytes, spermatids, and spermatozoa were placed in that order from the basal to the adluminal compartments. Fibroblasts, an average quantity of collagen fibers, blood vessels, and Leydig cells were present in the interstitial space, although the space was small. Primary spermatocytes were located near spermatogonia and had big, rounded central nuclei, whereas spermatogonia were basal locations and had small, spherical nuclei. Primary spermatocytes and spermatids were found nearby. Their elongated, strongly pigmented nuclei allowed for the identification of the elongated spermatids. Spermatogonia sitting on the basement membrane were found to be interspersed with Sertoli cells with oval nuclei (Figure 2). Polygonal or spherical Leydig cells with single or double nuclei and granular cytoplasm surrounding the blood arteries in the interstitial tissue were determined. These cells could be seen alone or in groups (Figure 2).

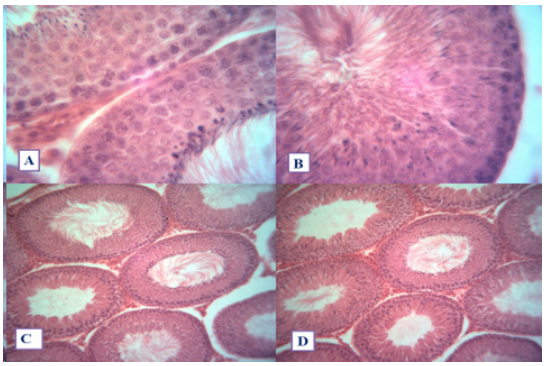

Fig. 2. *A normal connection of the germ cells and normal architecture of the interstitial tissue in a section of rat testicular tissue stained with H&E. Control group. A & B - 400x magnification; C and D - 200x magnification.*

Methotrexate caused testicular injury as evidenced by histopathologic examination, which showed seminiferous tubule degeneration as manifested by sloughing, atrophy, and germ-cell degeneration. The tubules' lining was disordered; most had a few layers of spermatogonia or a few layers of spermatocytes. The diameter of the tubules decreased. The uneven basal lamina surrounding each tubule reduced the height of the layers. The interstitial area was enlarged (Figure 3).

Numerous deteriorated eosinophilic cells with condensed chromatin mixed with apoptotic bodies and cell debris were seen in addition to vacuolar degeneration. Seminiferous tubule morphologies in the testicular tissue of the rats treated with methotrexate were irregular, and their diameters were significantly smaller than those of the control group (Figures 3 and 4). Compared to the rats treated with methotrexate alone, those treated with EPO significantly recovered the seminiferous tubule diameters. Additionally, the germinal epithelium had blatant disarray along with aberrant cellular attachment (Figure 4, Table 3). Most of the cells observed were spermatogonia cells. Multinucleated cells with two or three nuclei were also found in interstitial tissue. The interstitial connective tissue included an amorphous substance, which caused the connective tissues to break down noticeably and enlarge the interstitial tissue spaces (Figure 4).

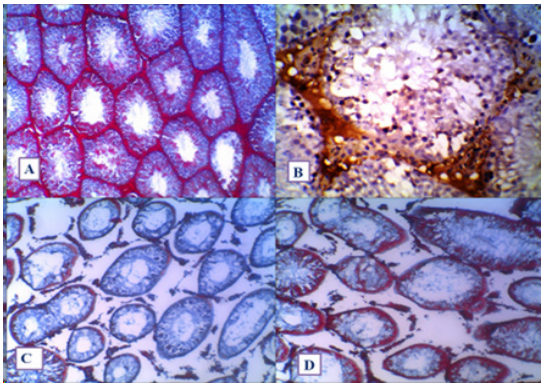

Fig. 3. *A: Collagen fibers are distributed normally in the interstitial tissue surrounding the seminiferous tubules in testicular tissue from the control group. Mallory's trichrome (200x magnification). B: Immunostaining for caspase-3 reveals a minimal expression in the seminiferous tubules' germinal cells. Control group (400x magnification). C and D: Methotrexate-treated tissue reveals a significant decrease in collagen fibers in the interstitial tissue surrounding the seminiferous tubules. Mallory's trichrome (200x magnification).*

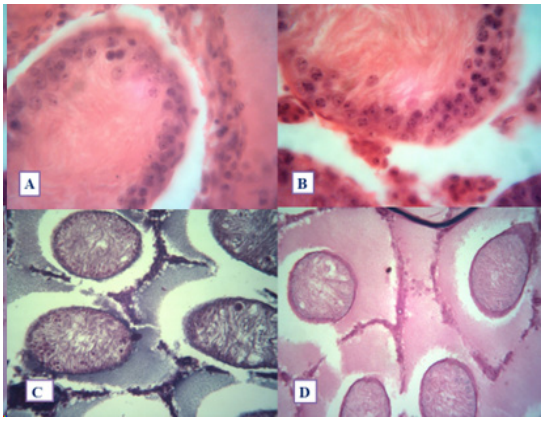

Fig. 4. *A and B: The germinal epithelium in the methotrexate-treated rat's H&E-stained piece is disorganized and uneven (400x magnification). C and D: Testicular tissue from a rat treated with methotrexate shows increased diameter of the interstitial spaces. (200x magnification). C) Mallory's trichrome (200x magnification). D) H&E staining (200x magnification)*

Compared to Group 2, Group3 displayed a large increase in collagen fibers and a significant recovery of the interstitial tissue to normal levels. Seminiferous tubules had a better organizational structure and were covered in conventional basement membrane. There were more spermatozoa tails in the lumina, fewer tubules had shed the germinal epithelium from their basement membrane, and the interstitial space was less than in Group 2. Better structured germinal epithelium-lined seminiferous tubules were found. The basement membrane supported the Sertoli cell. Wide intercellular gaps might be seen in some locations. There were not many Leydig cells with vesicular nuclei in the interstitial tissue (Figure 5).

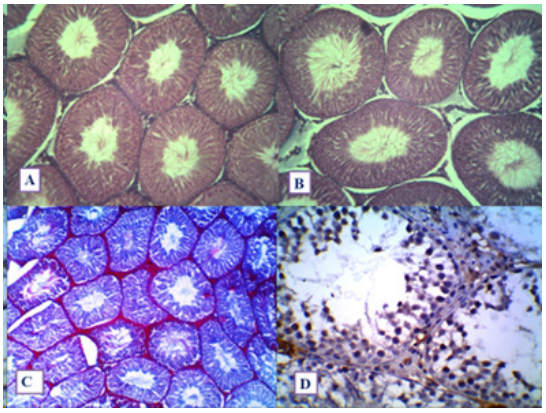

Fig. 5. *A and B: Testicular tissue from the EPO-treated rat has partial recovery to normal structure (H&E, 200x magnification); C: In comparison to the control group, testicular tissue treated with EPO exhibits a more or less identical distribution of collagen fibers in the tissue surrounding the seminiferous tubules. Mallory's trichrome (200x magnification). D: Caspase-3 immunostaining in germinal cells is moderately expressed in a region that has received erythropoietin treatment (400x magnification).*

The seminiferous tubules germinal epithelium was significantly reduced, underwent apoptotic alterations, was disorganized, and was depleted in Group 2, compared to the control group. Compared to Group 2, there was a markedly substantial recovery of the germinal epithelium in Group 3.

Table 3.

Histopathological changes in the testicles after nine weeks treatment in the study groups.

	Diameter of seminiferous tubules (μm)	Thickness of seminiferous tubules (μm)	Number of caspase-3+ cells (in 100 tubules	Area percentage of the collagen fibers
Group 1	179. 22 ± 14.11	100.76 ± 8.66	11.99 ± 2.11	139.11 ± 19.98
Group 2	132.57 ± 36.22	191.70 ± 31.49	38.55 ± 2.96	70.23 ± 17.76
Group 3	189.89 ± 11.87	89.42 ± 16.33	14.99 ± 3.99	151.89 ± 18.89
Statistic	F=33.753, P= 0.0000 P_{1-2}=0.0000 P_{1-3}=0.3285 P_{2-3}=0.0000	F=141.454, P= 0.0000 P_{1-2}=0.0000 P_{1-3}=0.2136 P_{2-3}=0.0000	F=435.751, P= 0.0000 P_{1-2}=0.0000 P_{1-3}=0.0097 P_{2-3}=0.0343	F=108.042, P= 0.0000 P_{1-2}=0.0000 P_{1-3}=0.0911 P_{2-3}=0.0000

The testicular tissue from the control testes had modest caspase-3 immunostaining and a small number of caspase-3-positive cells in the seminiferous tubules. Compared to the control group, the number of caspase-3-positive cells in the seminiferous tubules increased significantly in Group 2. Additionally, Group 3 showed a markedly significant increase in caspase-3-positive cells in the seminiferous tubules compared to the control group (Figure 5, Table 3).

Discussion

Our study showed that rats treated with methotrexate had testicular damage, which was demonstrated biochemically by decreased testosterone levels as well as several histological alterations. The adverse effects of cytotoxic medications like methotrexate, particularly reproductive damage reported in the numerous experimental studies, limit their effectiveness as anticancer therapies.[11] According to data from earlier investigations, there is sperm DNA damage, sperm count decline, and seminiferous tubule disorder.[12] Methotrexate toxicity causes cellular macromolecule damage by accumulating ROS and depleting antioxidants.[13] Various therapeutic agents have been tested for their ability to reduce testicular damage during chemotherapy. In animals, many tissues and organs, including the spinal cord, kidneys, liver, heart, lungs, brain, intestines, and retina, have been shown to suffer less ischemia damage when given EPO and sildenafil.[14]

Methotrexate has been investigated in several animal models, and dose, time, and animal species influenced drug toxicity. Previous studies have shown that exposure of male mice, like many other animals, to methotrexate results in similar adverse effects in the testes, including loss of testicular, seminal vesicle, and prostate weight, as well as morphological abnormalities of the testes.[15] In the methotrexate group without EPO, we observed degeneration of the seminiferous tubules, a significant decrease in spermatogenic cells, pyknotic nuclei, and cytoplasm vacuolization. These results may be because dihydrofolate reductase, an essential enzyme required for healthy DNA synthesis, was inhibited, preventing primary spermatocytes and spermatids from successfully replicating their DNA.[16] In our study, methotrexate caused seminiferous tubule degeneration, a significant drop in spermatogenic cells, pyknotic nuclei, and vacuolated cytoplasm. According to a recent study, Sertoli cells regulate the spermatogenic process; therefore, cytoplasmic vacuolation may be caused by damage to Sertoli cells.[17] In a study by Yucel et al.,[18] methotrexate administration increased histopathological damage, TAC (total antioxidant capacity), TOS (total oxidative status), and OSI (oxidative stress index) levels in hepatic tissue. Numerous studies have shown that EPO has cytoprotective properties in organs such as the brain, kidneys, heart, and retina.[19] Infusion of recombinant human EPO in a rat model of ischemic brain injury reduced neuronal death by reducing the levels of pro-inflammatory cytokines such as TNF-alpha, IL-6, and MCP-1.[20]

In conclusion, the present study shows that EPO has clear androgenic and antioxidant activities and reduces the adverse effects of methotrexate on testicular histology. These results also suggest further research into the use of EPO as a drug to protect patients from the adverse effects of chemotherapy.

Competing Interests

The authors declare that they have no competing interests.

Acknowledgments

This publication was supported by the Deanship of Scientific Research at Prince Sattam bin Abdulaziz University, Al-Kharj, Saudi Arabia.

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International Journal of Biomedicine 13(4) (2023) 356-359
http://dx.doi.org/10.21103/Article13(4)_OA21

ORIGINAL ARTICLE

Biotechnology

INTERNATIONAL JOURNAL OF BIOMEDICINE

Effect of Surface Treatment Method of Light-Cured Material on Its Toxic Properties

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Abstract

In recent decades, a large number of different nanocomposite materials have appeared, which have found wide application in almost all areas of life, including medicine. However, to date, the properties of these materials have not been completely studied. The evaluation of toxicity and biocompatibility is particularly relevant. In this regard, this study aimed to investigate the effect of the surface treatment method of light-cured material on its toxic properties for normal fibroblasts.

For this purpose, light-cured nanohybrid composite material Herculite XRV Ultra in the form of 10×5 mm plates (smooth and notched) were incubated with a culture of rat fibroblasts, after that, morphological changes were assessed, and "toxic exposure zones" (a distance from the test plate to a layer of intact fibroblasts) were measured. As a result, it was ascertained that although the investigated material has moderate toxicity to normal cells of the organism, the degree of the nanocomposite toxicity and, in particular, the size of the zone of toxic influence is significantly affected by the properties of its surface, depending on the mechanical action on the restorative material.**(International Journal of Biomedicine. 2023;13(4):356-359.)**

Keywords: nanocomposite • light-curing material • fibroblasts • cell culture • BioStation CT

For citation: Shurygina IA, Belozerceva OP, Trukhan IS, Dremina NN, Shurygin MG. Effect of Surface Treatment Method of Light-Cured Material on Its Toxic Properties. International Journal of Biomedicine. 2023;13(4):356-359. doi:10.21103/Article13(4)_OA21

Introduction

The development of nanocomposite materials is one of the most popular but not fully studied areas of nanotechnology. In recent decades, a great step forward has been made in the creation of particles ranging in size from 1 to 100 nm .[1] Nanoparticles, which can be both organic (liposomes, polymers) and inorganic (metals, metal oxide, ceramics), as well as carbon-based ones,[2] are used in various fields of activity, among which one of the main places is occupied by modern medicine.[3] At the same time, materials based on nanoparticles are utilized not only in diagnostic and therapeutic practice, but also in scientific areas of medicine.[4,5] These include nanocomposites for manipulating biological systems, nanoparticles heated by magnetic fields and used for thermal ablation or other delicate effects on cells, systems for targeted drug delivery without biological and chemical signals, and nanoparticles applied as contrast agents for biological imaging.[6]

For more than four decades, composite restoration materials have been used in dentistry[7] not only as a filling material, but also for the production of reliable dental structures and devices.[8] The leading positions among restoration materials are occupied by universal light-cured composites.[9] These materials have a number of advantages compared to chemically curing materials, which include resistance to chemical and thermal effects from the aggressive environment of the oral cavity, reliable connection of a hydrophobic composite and hydrophilic dentin, high physical and biochemical compatibility with dental tissues, and others.[10] The polymerization reaction of such composites is known to occur under the influence of light energy with a wavelength of 400-500 nm, i.e., visible blue light, which is considered the safest for living cells and tissues, and without the formation of by-products.[11,12] In this regard, currently, light-cured composites have almost completely replaced chemical fillings. In addition to filling, photopolymers

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are widely used for the restoration of teeth, the manufacture of composite veneers and inlays, for splinting of teeth in the periodontitis treatment, as well as repair of ceramic chips on crowns. However, despite their undeniable advantages, the safety issues of using these materials remain relevant. In this connection, in biomedical research, special attention is paid to the study of potential composite material toxicity to normal cells of the organism.

Thus, this study aimed to investigate the effect of the surface treatment method of light-cured material on its toxic properties for normal fibroblasts.

Material and methods

The primary culture of fibroblasts was obtained from the omentum of three-month-old male Wistar rats weighing 200–250 g. The experiments were performed in accordance with the norms for the humane treatment of animals, which are regulated by the International Guidelines of the Association for the Assessment and Accreditation of Laboratory Animal Care, following the protocol approved by the Institutional Animal Care and Use Committee of the Irkutsk Scientific Center of Surgery and Traumatology (Protocol No. 9 of 12/16/2021).

To obtain a pure culture, fibroblasts were subcultured every 7 days. After passage 3, the resulting pure culture of fibroblasts was used for experimental studies.[13,14]

For research, a modern light-curing nanohybrid universal composite material, Herculite XRV Ultra Intro Kit (KERR, USA), a combination of a polymer, barium glass nanoparticles, and a silicon nanofiller, was chosen. From this material, two plates with a size of 10 mm × 5 mm were formed: the first plate had a smooth surface, and the second one had grooves (notches) mechanically inflicted on its surface.

The resulting samples were placed in culture plates containing fibroblast monolayer cultures, and the morphological changes in cells were evaluated during 6 days of incubation. The "zone of toxic effect" was determined by measuring the distance from the plate to the fibroblast layer in microphotographs on days 2, 3, and 6.

Photodocumentation of the morphological changes in cells and assessment of the influence of samples with different surface characteristics on fibroblast culture were carried out using the BioStation CT 4.1 (Nikon) ("Diagnostic images in surgery"). The measurement of the "toxic effect zone" was performed with Nis-Elements AR, 4.1 (Nikon) software product. Statistical analysis was carried out in the R programming environment. The Wilcoxon-Mann-Whitney test was used for pairwise comparison.

Results

As a result of observations, it was found that a day after the introduction of samples with and without notches into the fibroblast culture, in both cases, the cell monolayer looked intact and was in contact with the surface of the plates. However, in the case of testing the notched sample, slight toxic granularity was observed among the fibroblasts adjacent to the plate surface (Figure 1).

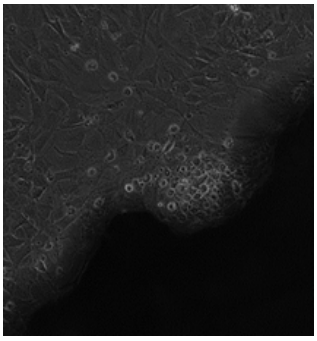
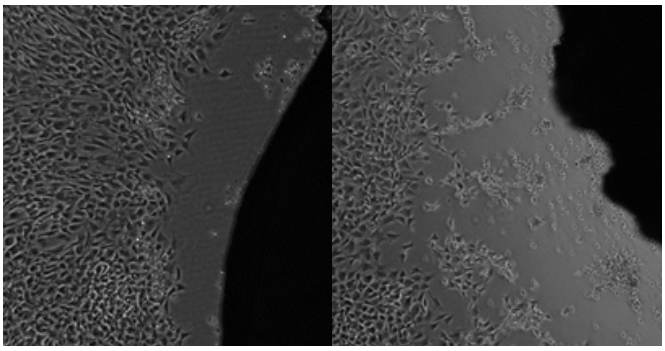


Fig. 1. *Fibroblast culture incubated with notched sample, 1 day after the start of the experiment. Phase contrast, 10x magnification.*

On the second day of the experiment, changes in cell morphology were observed, fibroblast detachment and the intact cell layer removal from the surface of the nanohybrid samples were recorded. With the sample without notches, cell detritus and altered fibroblasts were noted only near the sample surface, while the fibroblast monolayer looked intact.

When cells were incubated with notched samples, in the zone of contact with the plate, dead fibroblasts with toxic granularity were observed, followed by a layer of morphologically altered cells. During the subsequent incubation of fibroblast culture with the tested samples, the described effects continued to develop, and their progression was registered at 3 and 6 days (Figure 2A, 2B).



A B

Fig. 2. *Fibroblast culture incubated with samples without notches (A) and with notches (B), 3 days after the start of the experiment. Phase contrast, 4x magnification.*

When measuring the "zone of toxic influence," it was found that after days 2, 3, and 6, this zone was significantly wider for a sample with notches than without notches (Figure 3).

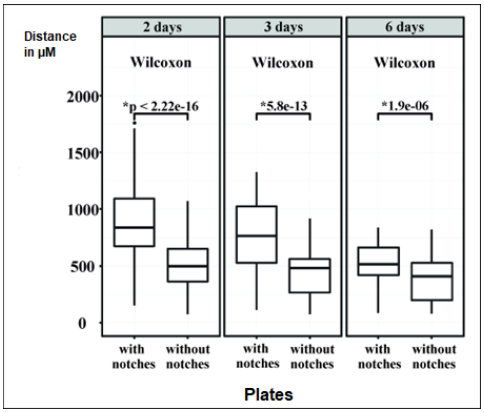


Fig. 3. *The distance between fibroblasts and plates with and without notches at various observation periods. The Wilcoxon-Mann-Whitney test.*

Discussion

The significance of nanocomposite materials with particle sizes from 1 to 100 nm is continuously increasing, as is the importance of such materials for medical practice. At the same time, it is known that the properties of synthesized materials depend mainly on the size and the characteristics of the functional surface of nanoparticles. In particular, the characteristics of nanocomposites, such as solubility, transparency, color, absorption or emission wavelength, conductivity, melting temperature, and catalytic behavior, can be controlled by changing the particle size. However, as the size decreases, the fraction of atoms found on the nanoparticle surface increases relative to their fraction inside the particle volume.[1] For example, a nanoparticle with a radius of 2.5 nm and a density of 5 g/cm^3 has an area of 240 m^2/g when assuming a spherical shape. Consequently, about 20% of the particle atoms are on its surface, which means that such nanoparticles can be more reactive.[15] In addition, due to high energy adhesive forces close to the surface, particles either agglomerate with each other or act as a filter to other small molecules, so changing the composition, size, or surface features can significantly modify the physical and chemical properties of nanoparticles whose components individually also have biological activity. Thus, the composition of light-cured materials includes, as a rule, fine-dispersed particles of silicon dioxide, pre-polymerized fillers, and organic matrix;[16] additional components are introduced to improve strength and reduce shrinkage to prevent degradation, as well as prolong the durability of the material.[17] At the same time, it is known that silicon is not only an indispensable element in the development of human bones and joints, but is also closely related to the immune system and connective tissue regulation. Silicon ions are able to induce the formation of calcium matrices, enhance bone regeneration through the ERK MAPK signaling pathway, and increase the secretion of type I collagen.[18] Some investigations have confirmed that silicon ions can activate the WNT pathway of bone marrow stromal cells, thus promoting the proliferation, differentiation, and expression of osteogenic proteins.

However, despite the unique physicochemical and biological properties of nanocomposite materials, not only their mechanical and aesthetic properties are important for their use in dental practice, but also their potential biocompatibility and non-toxicity for normal organism cells. For instance, the literature describes cases of adverse effects of light-cured material on biological tissues,[19,20] as well as a decrease in the viability of cell cultures, which is enhanced with increasing exposure time of the composite.[21] In the latter case, after 72 hours of incubation with the tested light-cured materials, the researchers observed more severe cytotoxic effects compared to the results after 24 hours of exposure. Additionally, it was found that after insufficient photopolymerization, the remaining free monomers are the main cytotoxic agents, and they can be released into the pulp tissue, which leads to inflammation and, ultimately, necrosis.[22] It should also be noted that the material strength depends not only on the proportion of inorganic components, but also on the particles' size, shape, and microstructure.[23]

In addition to the direct influence of the component composition of nanocomposite material, its toxicity and biocompatibility can be affected by such factors as the route of introduction and systemic distribution in the organism, as well as the properties of the surface in contact with living cells of the organism.[21,24] So, our study established a statistically significant dependence of the material toxicity on the roughness of its surface. It was demonstrated that the mechanical treatment of the sample, on the one hand, destroying the nanocomposite matrix integrity, and on the other hand, increasing the contact area of the material with the analyzed cells, makes a significant contribution to the toxic properties of the nanohybrid material Herculite XRV Ultra. Previously, sources have already noted that Herculite XRV Ultra has a high porosity,[25] which suggests an increase in the interaction area of the material with surrounding tissues; however, a decrease in its roughness after the polishing procedure was also demonstrated.[26] This indicates the need for careful polishing when using this composite material in dental practice. At the same time, it was found that imitation of exposure to the material identical to regular brushing of teeth during the year leads to an increase in its roughness,[27,28] which is also important to take into account, based on the changes in the toxic properties of the material established by us, depending on the surface unevenness. This study also indicates that the investigated filling material should not be under the gum and should not be in direct contact with gum tissue, since it has a cytotoxic effect on fibroblasts.

Conclusion

In this study, it was found that this light-cured nanocomposite has moderate toxicity to fibroblasts, and its toxic properties are determined not only by the chemical composition of the nanohybrid material, but also by the characteristics of its surface, depending on the mechanical influence on the restoration material. The method of toxicity assessment used in the study can be defined as universal for testing new materials, which are supposed to be applied in practice in a solid form insoluble in aqueous media.

Competing Interests

The authors declare that they have no competing interests.

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International Journal of Biomedicine 13(4) (2023) 360-363
http://dx.doi.org/10.21103/Article13(4)_ShC1

SHORT COMMUNICATION

INTERNATIONAL JOURNAL OF BIOMEDICINE

Direct Current Potential of Brain as a Stress Marker on Different Stages of Adaptation in Northern Climates

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Abstract

Background: The main changes in the parameters of functional systems in the process of adaptation occur in the first year of living in new conditions, and the main role in regulating human life support systems is played by the central nervous system. The brain's role in regulatory processes can be assessed by examining the levels of direct current potentials (DCP) in various brain areas by the Neuroenergy Mapping (NEM) method. The aim of this study was to assess the brain's DCP in a group of Indian students living in the Arctic region (Arkhangelsk city) for the first 6 months after arrival.

***Methods and Results*:** The study involved 106 young people aged 19–21 years. Registration, processing, and analysis of DCP were carried out in the following groups: 34 of the participants who lived in Arkhangelsk for less than 3 months at the time of the study (Group 1, short-term adaptation); 37 participants who lived in Arkhangelsk more than 6 months (Group 2, long-term adaptation); and in 35 participants who were born and are permanently residing in Arkhangelsk (Group 3, control). To study neurometabolism, the electrophysiological NEM method was used based on measuring the level of DCP. The highest functional activity among Indian students of Group 1 was found in the central (Cz) and parietal (Pz) regions. In Indian students of Group 2, on the contrary, a critically low total rate of DCP for the cerebral cortex was recorded, which indicates a decrease in the activity of the cerebral cortex. Potential values in all departments of the right hemisphere were recorded higher than the corresponding values of the left hemisphere. In Group 3, which included students living for a long time in extreme climatic and geographical conditions of the Arctic zone, there was also a stable right-hemispheric dominance with a predominance of activity in the central, parietal, and occipital regions of the cerebral cortex. The total value of DCP in the whole cortex was within the normal range, which indicates well-formed mechanisms of adaptation to extreme climatic conditions of the Arctic zone.

Conclusion: Indian students, depending on the time spent in the climatic conditions of the Arctic Circle, reveal various features of cerebral metabolism. Thus, the acute stage of adaptation to a cold climate shows inadequate responses to stress caused by new environmental conditions, such as a significant increase in DCP throughout the cerebral cortex with a maximum in the central leads. After 6 months of living in a new climate, the DCP level decreases, which indicates signs of an adaptive disorder of brain function.**(International Journal of Biomedicine. 2023;13(4):360-363.**

Keywords: direct current potential • brain • Arctic climate • adaptation

For citation: Kiryanov AB, Gribanov AV, Kozhevnikova IS, Anikina NYu, Alikberova MN. Direct Current Potential of Brain as a Stress Marker on Different Stages of Adaptation in Northern Climates. International Journal of Biomedicine. 2023;13(4):360-363. doi:10.21103/Article13(4)_ShC1

Introduction

The process of adaptation in new climatic conditions generally places the strongest stress on the functional systems of the body, with difficult compensation,[1,2] and often has a compromise nature, i.e., ensuring the effectiveness of some physiological systems leads to a decrease in the efficiency of regulation of others.[2,3] Adaptability is achieved by reducing some of the body's resources. The stage of short-term adaptation to the specific conditions of the Arctic is characterized by increased anxiety, reduced mental and physical performance, boundary shifts in hormonal status, and activation of emotional information

in memory processes.[4,5] Long-term persistence of this status increases the risk of developing diseases. The duration of this stage, in which the compensatory and regulatory mechanisms of homeostasis are destabilized, according to most sources, takes about six months.

In the short-term adaptation process, the body's homeostatic systems are activated, and two opposite processes occur. On the one hand, when the sympathetic part of the nervous system is activated, catabolic reactions occur, which provide the body with the necessary energy. On the other hand, a wide range of neurohormonal factors directly affect metabolism and activate protein synthesis in various organs and tissues of the body.[3,5,6]

The age period of 18-21 years is characterized by both the maximum adaptive reserves of the body and the greatest psychological stress. Often, the beginning of studies at a university or college is accompanied by a move to new regions with dramatically different climatic conditions from the usual ones. It is known that the main changes in the parameters of functional systems in the process of adaptation occur in the first year of living in new conditions, and the main role in regulating human life support systems is played by the central nervous system.[7,8] The brain's role in regulatory processes can be assessed by examining the levels of direct current potentials (DCP) in various brain areas by the Neuroenergy Mapping (NEM) method.[7,9] This method is cheap and non-invasive and may be used in any subject independent of health, age, and gender. NEM allows us to visualize the metabolic process in different brain structures in various clinical situations. The DCP level correlates with various biochemical parameters of the brain tissues, allowing resulting data to be used for diagnosing various pathophysiological conditions.

The aim of our study was to assess the brain's DCP in a group of Indian students living in the Arctic region of Russia (Arkhangelsk city) for the first 6 months after arrival.

Materials and Methods

The study involved 106 young people aged 19–21 years. We formed comparison groups to level out the age and gender characteristics of the subjects for an objective assessment of differences in metabolism and infraslow activity of the cerebral cortex at short-term and long-term stages of adaptation to new socio-climatic conditions.

In our study, registration, processing, and analysis of DCP were carried out in the following groups: 34 of the participants who lived in Arkhangelsk for less than 3 months at the time of the study (Group 1, short-term adaptation); 37 participants who lived in Arkhangelsk more than 6 months (Group 2, long-term adaptation); and in 35 participants who were born and are permanently residing in Arkhangelsk (Group 3, control).

To study neurometabolism, the electrophysiological NEM method was used based on measuring the level of DCP. DCP was recorded in the first half of the day, in a room with a comfortable air temperature; the time from the last meal was at least 2 hours, and the subjects were in a state of emotional rest. The study was conducted on a hardware-software complex for topographic mapping of the electrical activity, with 12 channels for recording DCP. The active electrodes were placed on the head using a 10×20 pattern; the reference electrode was placed on the left wrist. DCP processing was carried out using specially developed, licensed NEK-12 software. The values given in the study represent the average DCP level.

Statistical analysis was performed using the statistical software IBM SPSS Statistics 23. The normality of the distribution of traits in the groups was assessed using the Shapiro-Wilk criterion. Median (Me) values were presented with interquartile (IQ) ranges (IQR; 25th to 75th percentiles). Mann-Whitney U test was used to compare differences between two independent groups. A value of $P<0.05$ was considered significant. A Bonferroni correction was used to adjust for multiple testing, with a significance criterion of $P<0.017$ used for each primary analysis (i.e. 0.05/3).

The study was approved by the Ethics Committee of the Northern State Medical University (Arkhangelsk). Written informed consent was obtained from all participants.

Results and Discussion

The DCP levels for the cerebral cortex topographic areas in the study groups are presented in Table 1. According to the results obtained, the highest functional activity among Indian students of Group 1 was found in the central (Cz) and parietal (Pz) regions, which coincides with the normative dynamics of DCP in the cerebral cortex. The trend of transition was to right-hemispheric dominance: The functional activity of the parietal and temporal regions in the right hemisphere began to predominate. At the same time, the greatest activity in the frontal and central regions of the cortex was recorded in the left hemisphere. The higher total indicator of DCP in Group 1 indicates increased activity of cortical centers at the initial adaptation stage.

In Indian students of Group 2, on the contrary, a critically low total rate of DCP for the cerebral cortex was recorded, which indicates a decrease in the activity of the cerebral cortex. The functioning of any physiological system of vital activity is of a compromise nature. Removal of tension from the cerebral cortex indicates a more active inclusion of subcortical structures at the long-term stage of adaptation. The highest values are observed in the central, right-central, and right-parietal leads (Cz, Cd, Pd). In this case, the frontal structures are turned on, which is indicated by a relatively high level of potential, compared with other values, in the frontal (Fz) lead. The neurons of the parietal and frontal associative fields form the mechanisms of short-term memory, the key element of which is intracortical reverberation. As a result of this, there is probably a sharp decrease in the potential of other areas of the cerebral cortex with the transition of activation to subcortical structures. In addition, in the sixth month of their stay in the new climatic and geographical conditions, students formed a stable right-hemispheric dominance. Potential values in all departments of the right hemisphere were recorded higher than the corresponding values of the left hemisphere. Several authors suggest the presence of adaptation centers in the right hemisphere. In addition, it is known that it is the right hemisphere responsible for regulating the endocrine glands, as well as immunity. It is more autonomous and less subject to various corrective influences.

Table 1.

The DCP levels (mV) for the cerebral cortex topographic areas in the study groups.

Lead	Group 1 [1]	P_{1-2}	Group 2 [2]	P_{2-3}	Group 3 [3]	P_{1-3}	Norm
Fpz	11.08 (3.21; 22.64)	0.021	5.22 (-2.79; 12.41)	0.128	9.05 (2.69; 15.35)	0.080	8.80
Fd	12.28 (1.06; 22.85)	<0.001	-0.11 (-5.75; 8.64)	0.022	8.01 (-0.05; 14.66)	0.166	5.80
Fs	16.09 (4.16; 23.87)	<0.001	-1.45 (-6.15; 8.64)	0.001	7.46 (-0.01; 15.56)	0.159	7.20
Cd	16.55 (5.88; 32.04)	<0.001	8.66 (-1.31; 19.31)	0.245	15.78 (6.69; 21.83)	0.734	9.00
Cz	21.99 (9.09; 34.76)	<0.001	8.57 (1.56; 18.62)	0.028	15.81 (7.10; 25.19)	0.253	12.00
Cs	16.99 (4.45; 29.63)	<0.001	2.64 (-7.17; 14.83)	0.020	8.84 (4.43; 19.19)	0.089	9.00
Pd	16.97 (5.49; 29.58)	<0.001	6.40 (-2.05; 17.39)	0.041	14.43 (5.41; 20.18)	0.072	9.50
Pz	21.61 (7.85; 29.86)	<0.001	2.37 (-5.46; 12.13)	0.002	11.62 (4.16; 20.05)	0.027	10.90
Ps	16.07 (4.57; 23.77)	<0.001	5.36 (-2.53; 19.91)	0.025	8.81 (3.81; 18.07)	0.093	9.50
Oz	17.87 (11.62; 29.27)	<0.001	2.82 (-4.91; 12.09)	0.003	11.60 (4.55; 17.30)	0.010	9.60
Td	16.18 (2.37; 25.78)	<0.001	1.42 (-4.51; 10.70)	0.003	10.98 (2.05; 18.34)	0.143	9.00
Ts	14.99 (4.57; 24.85)	<0.001	0.42 (-5.92; 10.35)	0.013	7.60 (0.51; 15.39)	0.021	10.50
Sum	222.07 (56.68; 328.55)	<0.001	39.69 (-29.50; 171.91)	0.004	112.91 (75.92; 196.38)	0.884	111.10

"Norm" - DCP levels obtained from young people of a comparable age group living in the climatic conditions of Central Russia. Fpz - frontal central lead, Fd - right frontal lead, Fs - left frontal lead; Cz – central lead, Cd - right central lead, Cs - left central lead; Pz - central parietal lead, Pd - right parietal lead, Ps - left parietal lead; Oz - occipital lead; Td - right temporal lead, Ts - left temporal lead.

In Group 3, which included students living for a long time in extreme climatic and geographical conditions of the Arctic zone, there was also a stable right-hemispheric dominance with a predominance of activity in the central, parietal, and occipital regions of the cerebral cortex. The total value of DCP in the whole cortex was within the normal range, which indicates well-formed mechanisms of adaptation to extreme climatic conditions of the Arctic zone.

One of the signs of the normal distribution of DCP in the cerebral cortex is the principle of "dome," in which there is a gradual decrease in DCP in the sagittal and transverse planes from the maximum values in the vertex region and increased values in the leads of the left hemisphere, compared to the right.[7] The distribution of DCP in the studied groups is shown in Figure 1. As can be seen, the dome principle is violated to a greater or lesser extent in all groups. Indian students in short-stage adaptation had high values of DCP in the central and parietal regions with a sharp decrease toward the periphery. Indian students in the long-term adaptation had a bias in the maximum DCP values in the frontal region and a decrease in DCP in the parietal and occipital regions. Among northern students, the violation of the principle of "dome" was manifested in the shift of higher rates of DCP to the right hemisphere. Higher DCP values in the right leads, according to the studies of Gribanov et al.,[4,10,11] are a distinctive feature that characterizes the redistribution of the DCP level in people living in the North.

It is now known that humans living in uncomfortable climatic conditions are accompanied by an increased functioning of certain body systems. With insufficient autonomous support for the organism's life, the mobilization of strategic reserves is carried out with the participation of central regulatory mechanisms.[12]

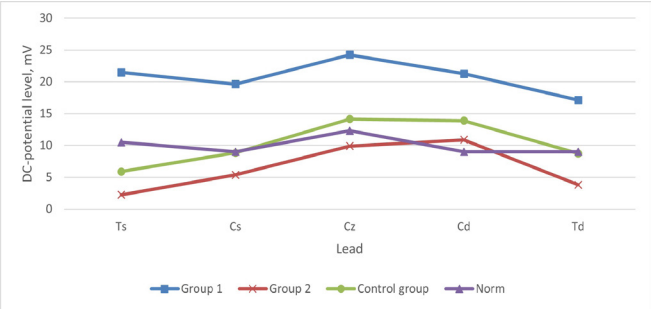

Fig. 1. *DCP distribution in coronal plane.*

Conclusion

Thus, Indian students, depending on the time spent in the climatic conditions of the Arctic Circle, reveal various features of cerebral metabolism. Thus, the acute stage of adaptation to

a cold climate shows inadequate responses to stress caused by new environmental conditions, such as a significant increase in DCP throughout the cerebral cortex with a maximum in the central leads. After 6 months of living in a new climate, the DCP level decreases, which indicates signs of an adaptive disorder of brain function.

Competing Interests

The authors declare that they have no competing interests.

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International Journal of Biomedicine 13(4) (2023) 364-366
http://dx.doi.org/10.21103/Article13(4)_ShC2

SHORT COMMUNICATION

INTERNATIONAL JOURNAL OF BIOMEDICINE

Analytical Performance of Direct Rapid Nucleic Acid Assay for Detection of SARS-Cov-2

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Abstract

Background: A rapid and accurate test to detect SARS-CoV-2 is essential for controlling the transmission of the COVID-19. Rapid diagnostic tests are currently marketed, although it is uncertain how well they perform in actual clinical settings and with relevant subpopulations. We evaluated the clinical performance of the Direct Detect® SARS-CoV-2 Detection Kit (Coyote Bioscience Co., Ltd., Beijing, China) rapid, molecular-based assay.

Methods and Results: The clinical laboratory received 707 clinical samples for rapid PCR between December 2021 and March 2022, including confirmed or suspected COVID-19 cases. These samples were tested by the Direct Detect® SARS-CoV-2 Detection Kit and by the LabGun® COVID-19 ExoFast RT-PCR Kit. Of 707 specimens tested, 649(91.79%) were negative and 58(8.20%) were positive. The sensitivity and specificity of the rapid RT-PCR test were 79.31% (95% CI: 66.65% to 88.83%) and 99.54% (95% CI: 98.66% to 99.90%), respectively.

Conclusion: The Direct Detect® SARS-CoV-2 Detection Kit evaluated in this study was able to detect SARS-CoV-2 infection with high viral loads but not so for higher loads. To determine strategies for appropriate use, more investigation of the assay's field performance in various conditions is required.**(International Journal of Biomedicine. 2023;13(4):364-366.)**

Keywords: SARS-CoV-2 • molecular-based assay • RT-PCR • rapid tests

For citation: Jagan OA, Vellamkott MMP, Bava A, Anish J, Acharya V, Shakir M, Oommen S. Analytical Performance of Direct Rapid Nucleic Acid Assay for Detection of SARS-Cov-2. International Journal of Biomedicine. 2023;13(4):364-366. doi:10.21103/Article13(4)_ShC2

Introduction

The COVID-19 pandemic evolved to hold a significant influence on human health and life around the globe. The diagnostic standard for testing SARS-CoV-2 is a real-time reverse transcription polymerase chain reaction (RT-PCR) assay.[1] This technique, however, is time-consuming (the result might take up to 24 hours) and requires technically skilled personnel and special laboratories.[2]

The extraction and amplification done in a closed system in molecular testing give a minimal chance for false positives; well-trained personnel, sample type, and quality, or reagent kit quality are some of the many factors that provide a false-negative result despite the high sensitivity of the real-time PCR assay. Hence, analytical sensitivity plays a crucial role in the accuracy of COVID-19 diagnosis in a patient. Currently, a significant number of SARS-CoV-2 RT-PCR diagnostic tests are being widely utilized throughout the world, all of which claim to have different analytical sensitivities. Numerous studies compare the analytical sensitivity of various assays.[3,4]

As a result of the rapidly spreading COVID-19 epidemic, the FDA authorized the use of various molecular assays for in vitro diagnosis.[5] The accuracy of laboratory-based PCR testing combined with the convenience and speed of point-of-care (POC) rapid antigen testing would make for the ideal diagnostic for COVID-19.[6]

Commercially available rapid diagnostic assays for SARS-CoV-2 detection are simple and affordable; however, how well they perform in the real world is unknown. In this study, we tested performance characteristics of the Direct Detect® SARS-CoV-2 Detection Kit (Coyote Bioscience Co., Ltd., Beijing, China) rapid, molecular-based assay.

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Materials and Methods

A retrospective study was conducted at our hospital's Molecular Biology Laboratory between December 2021 and March 2022. The Institutional Review Board (BH/REC/025/22) and the Abu Dhabi Health Research and Technology Ethics Committee - Department of Health (DOH/CVDC/2022/1641) reviewed and approved the project. As per standard hospital procedure, general consent was obtained to collect data for research purposes.

This study included all the participants who underwent rapid PCR testing. The samples were kept at -80°C and retested using the LabGun® COVID-19 ExoFast RT-PCR Kit, the study's reference standard (Lab Genomics, Korea).

Direct Detect® SARS-CoV-2 Detection Kit Assay

Rapid Nucleic Acid Assay for SARS-CoV-2 was performed using a Direct Detect® SARS-CoV-2 Detection Kit. The Coyote Direct Detect® SARS-CoV-2 Detection kit detects the ORF1ab and N genes with an approximate run time of 40 min. The results are interpreted as positive if the cycle threshold (Ct) values of both the ORF1ab and N genes are ≤27; there is no significant amplification curve or if the Ct value is>27, it is considered negative. Repeat testing is recommended if a single gene is positive. The kit has an internal reference gene RNase P to monitor sampling and identify possible RNA transcription and PCR amplification inhibition.

Standard: RT-PCR Assay Kit

The LabGun® Exofast COVID-19 RT-PCR Kit is a real-time assay that detects the N and RdRp genes of the SARS-CoV-2 virus along with human RNase P, which was used as the internal control from human patient samples. This Standard is a CE-IVD, standardized and validated in-house for routine diagnostic of SARS-CoV-2 detection. Roche MagNA Pure 96 DNA and Viral NA Small Volume Kit was used to isolate and purify nucleic acids from nasopharyngeal swabs. The isolated nucleic acid was amplified directly on the Applied Biosystems QuantStudio® 5 Dx Real-Time PCR System using this kit.

We calculated the sensitivity (Se), specificity (Sp), positive predictive value (PPV), and negative predictive value (NPV) to determine the diagnostic value of Direct Detect® SARS-CoV-2 Detection Kit Assay.

Statistical analysis was performed using statistical software package SPSS version 23.0 (SPSS Inc, Armonk, NY: IBM Corp). A probability value of $P<0.05$ was considered statistically significant.

Results

A 2×2 table was drawn up based on the test positivity in both assays (Table 1). Reference real-time PCR diagnosed COVID-19 in 58 patients, of which only 46 were detected by the rapid PCR. Compared to the RT-PCR reference kit, the sensitivity and specificity of the Rapid PCR were 79.31% (95% CI: 66.65% to 88.83%) and 99.54% (95% CI: 98.66% to 99.90%), respectively (Table 1).

Table 1 summarizes the Direct Detect® SARS-CoV-2 Detection Kit test performance characteristics. The test result was stratified by the Ct values, and the highest sensitivity was observed for RT-PCR Ct values <30 and reduced substantially at Ct values >30, $P=0.0003$ (Table 2). Figure 1 represents the Ct values of all discordant RT-PCR specimen results in relation to the results of the Direct Detect® SARS-CoV-2 Detection Kit.

Table 1.

Clinical performance evaluation results of the Direct Detect® SARS-CoV-2 Detection test.

Comparison of Direct Detect® SARS-CoV-2 Detection test and reference RT- PCR				
		RT-PCR Results		Total
		Negative	Negative	
Direct Detect™ SARS-CoV-2 Detection Kit	Positive	46	3	49
	Negative	12	646	658
TOTAL		58	649	707

Performance characteristics of the Direct Detect® SARS-CoV-2 Detection test		
STATISTICS	Value	95% CI
Sensitivity	79.31%	66.65% to 88.83%
Specificity	99.54%	98.66% to 99.90%
Positive Likelihood Ratio	171.57	55.06 to 534.66
Negative Likelihood Ratio	0.21	0.13 to 0.34
Disease prevalence (*)	8.20%	
Positive Predictive Value (*)	93.87%	83.10% to 97.95%
Negative Predictive Value (*)	98.18%	97.02% to 98.89%
Accuracy (*)	97.88%	96.53% to 98.81%
(*) These values are dependent on disease prevalence.		

Table 2.

***Sensitivity of the Direct Detect® SARS-CoV-2 Detection test stratified by RT-PCR cycle threshold (Ct)* intervals.**

RT-PCR Ct value	n	Rapid PCR Positive	Sensitivity % (95% CI)	Rapid PCR Negative	False-Negative Rate (%)
≤ 30	34	30	88.24% (72.55% to 96.70%)	4	11.76
30-<40	24	16	66.67% (44.68% to 84.37%)	8	33.33

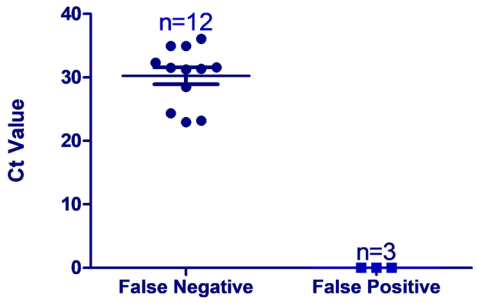

Fig. 1. *Discordant analysis between Direct Detect™ SARS-CoV-2 rapid PCR test and RT-PCR cycle threshold (Ct).*

Discussion

As the SARS-CoV-2 pandemic continues to persist, the disparity between the number of tests required and the testing capacity of laboratories or primary-care settings increases.[7] The ability to detect infected patients in a timely manner has been critical for viral infection control. POC tests have considerably decreased test result lag times, enabling faster clinical intervention and preventative action. There are not enough validation studies to back up the use of these POC tests in various patient scenarios, even though they show potential for usage as a component of a larger strategy for COVID-19 diagnosis and control.[8] This retrospective study comprehensively and systematically evaluated the clinical performance of the Direct Detect® SARS-CoV-2 Detection Test Kit.

As expected, our analysis of the data revealed that false-negative results were seen for high Ct values, whereas we noticed concordance between the POCT and RT-PCR tests at lower Ct, highlighting the potential of the POC test to detect more effectively high viral loads in subjects who were likely to be having symptoms.[9]

Across all 707 tested subjects, there were 12 false-negative results with Ct values between 22 and 40, and three false-positive results for RT-PCR negative results (Figure 1). A single negative test does not rule out infection in individuals because, as indicated for both RT-PCR and rapid PCR testing, the possibility of false-negative results exists due to either sample variability or viral load variation. Repeat testing is recommended if the initial test is negative and if symptoms persist and COVID-19 is suspected. Overall, the rapid PCR test revealed a moderate sensitivity (79.31%) and good specificity (99.54%) in our study compared to the manufacturer-reported sensitivity of 95.02% and a specificity of 99.33%.

However, the data showed a lower sensitivity than the sensitivity of an efficient POC test that the WHO recommended.[8] Data disparity may result from testing samples that were in the late stages of the disease, which may also be a contributing factor. When interpreting the results of POC tests in these samples, caution must be taken because SARS-CoV-2 infection affects a significant section of the population with an asymptomatic presentation.

Since our findings originated from a sizable cohort of participants tested regardless of clinical presentation, the primary limitation of the current investigation is the lack of clinical data. As a result, we are unable to correlate the sensitivity of the POC test with the beginning of symptoms. Recent genetic SARS-CoV-2 virus variants with mutations require close monitoring to assess the potential impact on POC testing.

Acknowledgements

We are thankful to Ms. Shamyla Siddique and Dr. Khaled Musallam for their constant cooperation and support throughout the work. We would like to thank Mr. Mayur Sabhani, Group Director - Laboratory Services, who provided all the facilities for this project in CoLAB, Burjeel Medical City.

Competing Interests

The authors declare that they have no competing interests.

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International Journal of Biomedicine 13(4) (2023) 367-370
http://dx.doi.org/10.21103/Article13(4)_CR1

CASE REPORT

INTERNATIONAL JOURNAL OF BIOMEDICINE

Acute Transverse Myelitis with Right Arm Paralysis in a Pediatric Patient: A Rare and Challenging Case Report

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Abstract

Acute transverse myelitis (ATM) is a rare inflammatory spinal cord disorder with varied clinical manifestations, particularly in the pediatric demographic. This report details the case of a 3-year-old child from Dibra, Albania, admitted with fever and sudden paralysis of the right arm. Initial symptoms included fever, fatigue, and anorexia, leading to total paralysis of the right arm. Laboratory findings highlighted elevated neutrophils at 66.6%. Crucially, an MRI of the spine indicated inflammatory lesions in the cervical region, confirming the diagnosis of ATM. The child underwent a comprehensive treatment regimen, including antibiotics, immunoglobulins, antiviral medications, corticosteroids, and physiotherapy. Post-treatment, while there was a marked improvement in the child's general health and a halt in the progression of the disease, significant motor deficits persisted in the right arm. Despite the severity of ATM and challenges in treatment, early intervention showcased a positive impact on the patient's health, emphasizing the critical nature of prompt diagnosis and treatment in pediatric ATM cases. This case offers insight into the clinical presentation, diagnosis, and management of ATM in young children, shedding light on a rare yet impactful neurological disorder.**(International Journal of Biomedicine. 2023;13(4):367-370.)**

Keywords: acute transverse myelitis • young children • MRI • treatment

For citation: Shehu A, Pilika K, Pilika A, Bushati A, Tako A, Aleksi K, Prifti E, Hysenaj B, Shpuza A. Acute Transverse Myelitis with Right Arm Paralysis in a Pediatric Patient: A Rare and Challenging Case Report. International Journal of Biomedicine. 2023;13(4):367-370. doi:10.21103/Article13(4)_CR1

Introduction

Acute transverse myelitis (ATM) represents an inflammatory disorder of the spinal cord with significant implications for patient health, especially in pediatric populations. The rarity of this disease in children makes understanding its nuances imperative for timely diagnosis and treatment.

The evidence has highlighted that the prevalence of ATM in the pediatric population stands at around 1-2 new cases per million children each year.[(1)] Timely detection of the disorder is critical due to the potential for rapid neurological deterioration when untreated.

A majority of ATM cases in children can be traced back to a preceding viral or bacterial infection. Pidcock et al. reported that roughly one-third of pediatric patients with ATM had experienced an illness or had recently been vaccinated prior to the onset of symptoms, suggesting a possible post-infectious or post-immunization etiology.[(2)] Infections leading to ATM include but are not limited to West Nile virus, herpes viruses, enteroviruses, HIV, Zika virus, Lyme neuroborreliosis, human T-cell leukemia virus type 1, *Mycoplasma* spp., and *Treponema pallidum*.[(3)] However, the exact cause of ATM remains largely unknown, and further exploration into its origins is warranted.

The spectrum of clinical manifestations of ATM is broad, and the symptoms can range from milder presentations,

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such as back pain, to severe cases of paralysis.[4] This diversity in clinical presentation underscores the importance of a comprehensive diagnostic approach, as exemplified in our current case of the 3-year-old child with ATM.

MRI is an indispensable tool in the diagnostic arsenal for ATM. Different studies have underscored that MRI findings, which reveal inflammation of the spinal cord, particularly in the cervical region, are crucial for a confirmed diagnosis of ATM.[5,6]

Therapeutically, the administration of high-dose corticosteroids emerges as the primary treatment method, a stance supported by research.[7] In addition to pharmacological interventions, physiotherapy has shown promise in aiding the recovery of motor functions in patients.

While ATM in children is uncommon, it commands significant clinical attention due to its potential to severely impact health outcomes. Effective diagnosis and early treatment interventions are vital for ensuring favorable patient outcomes.

Case Presentation

***Presenting Complaints*:** The child was admitted to the Infectious Disease Clinic (IDC), University Hospital Centre "Mother Teresa," Tirana, on 02.11.2022 with the diagnosis of febrile state and right arm paresis. On admission, the patient manifested symptoms of fatigue, vomiting, fever up to 38.5°C, and an inability to move the right arm.

***Disease History*:** According to the family, the child had been symptomatic for approximately three days before admission. Initial symptoms included fever (38.5°C), fatigue, anorexia, and subsequently, an inability to move the right arm (total paresis). Mild back pain was also reported. This constellation of symptoms prompted the family to seek medical attention at the IDC. Routine laboratory examinations were conducted. Notably, neutrophils were elevated at 66.6%. A chest X-ray revealed bilateral peribronchitis. Echography of the right shoulder and elbow joints was reported as normal. An antibiotic therapy regimen and infusions were initiated.

Post consultation with a pediatric neurologist, it was suggested to carry out MRI scans of the spine and head. The spine MRI displayed areas of hyperintense signal in the cervical spinal cord, indicative of inflammatory lesions (myelitis) (Figures 1 and 2).

Considering the patient's clinical condition and corroborative imaging findings, therapy with high-dose Methylprednisolone was initiated for seven days. Additionally, the child was treated with IgG for five days, Aciclovir, Ceftazidime, Omeprazole, infusions, and physiotherapy. Subsequent observations highlighted an overall improvement in the child's general condition and halting of the disease's progression. Remarkably, during the initial examination, no impairments in vital functions or sphincter functions were observed, and patellar and sensory reflexes were intact. At the end of in-hospital treatment, which included physiotherapy, the child showed slight mobility in the right arm, expected to continue improving with outpatient therapy.

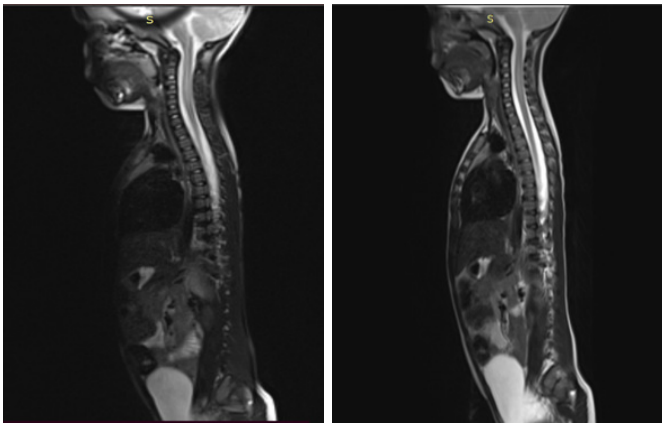

Fig. 1&2. *MRI scans of the spine and head before the initiation of the treatment.*

Increased T2 signal and expansion of the chord are seen extending between C7 and T12. The T2 signal abnormality involves central grey matter and dorsal columns. Linear sagittally oriented enhancement is seen posteriorly within the cord in the mid and lower thoracic cord.

Post-hospitalization, the child was prescribed a tapering dose of Prednisolone for four weeks, oral Omeprazole, oral Aciclovir for a week, and continuation of physiotherapy. Three months later, a follow-up MRI of the spine was advised by the neuroradiologist, which displayed no radiological sequelae (Figures 3 and 4).

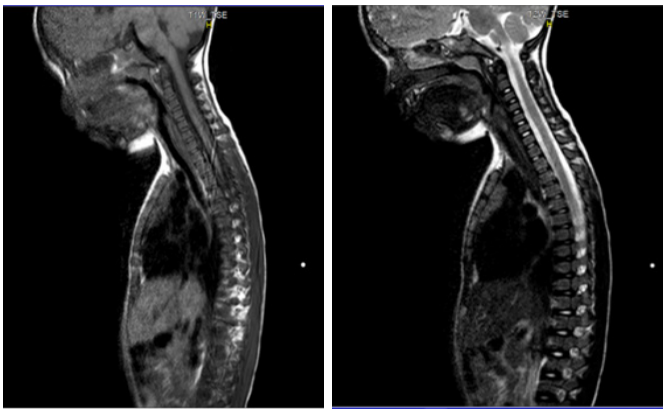

Fig. 3&4. *MRI scans of the spine and head after the completion of treatment.*

However, clinically, the right arm still exhibited a pronounced motor deficit despite ongoing physiotherapy. An electromyography (EMG) of the right arm, conducted in March 2023, indicated the absence of motor parameters registration in the upper right side, while sensory parameters were within normal limits.

Family History: The family denied any history of neurological disorders in close relatives.

Physical Examination: Upon examination, the child appeared stable and responded appropriately for his biological age. Mild headaches and transient dizziness upon standing were reported. Reflexes were present in both extremities. The examination revealed a pronounced deficit in the right arm,

characterized by restricted mobility, inability to raise the arm or move the fingers, and an absence of the right cubital reflex. However, sphincter functions were well-controlled. Cardiac auscultation presented clear tones. Lung auscultation showed vesicular breathing. The abdomen was soft, with the liver and spleen within normal limits. The child's blood pressure was recorded at 120/80 mmHg.

Laboratory Investigations: A detailed blood panel was conducted. Notable findings included elevated neutrophil count at 66.6%, a slight elevation in ESR (16 mm/h), and other parameters that largely remained within normal limits.

Imaging Findings: The MRI of the spine revealed hyperintense signals in the cervical region, suggesting inflammatory lesions typical of myelitis. Specifically, the areas of hypersignal were located in the cervical region and the medullary cone. These findings were instrumental in solidifying the diagnosis. In contrast, the MRI of the head displayed no abnormalities.

A chest X-ray further supported the clinical presentation, revealing bilateral peribronchitis. An echography of the right scapulohumeral and cubital articulation was performed to rule out any local joint or soft tissue pathologies that could be contributing to the right arm paresis. The results were within normal parameters, eliminating orthopedic causes.

Treatment Course: In response to the myelitis diagnosis and to counteract the inflammatory process, high-dose intravenous Methylprednisolone (400 mg daily) was administered for seven days. Antiviral therapy was provided in the form of Aciclovir (150 mg, three times daily), given the potential viral etiologies of myelitis. The child also received Ceftazidime, an antibiotic to manage potential bacterial infections that might be contributing to the febrile state.

To support the child's overall health and prevent gastric complications from high-dose corticosteroids, intravenous Omeprazole (20 mg, twice daily) was provided. IgG was administered for five days to modulate the immune response and offer potential therapeutic benefits in inflammatory conditions. Daily infusions of 0.9% sodium chloride and 5% glucose solution were given to maintain hydration and energy. Vitamin therapy was provided to support the child's nutritional needs, given the anorexic state. Moreover, physiotherapy was introduced early during the in-patient phase to encourage motor function recovery.

Post-Hospitalization Management: Upon discharge, outpatient management was crucial to ensure continued recovery. The child was prescribed oral Prednisolone, which was to be tapered over four weeks. This step was essential to reduce inflammation further while managing potential rebound effects. Aciclovir syrup was continued for a week to ensure complete antiviral coverage. Oral Omeprazole was given as a gastroprotective agent against potential gastric ulcers from prolonged steroid use. Emphasis was also placed on continuing physiotherapy to aid in the gradual recovery of right arm function. The introduction of vitamin therapy was maintained to ensure adequate nutrition.

Follow-up and Prognosis: A subsequent MRI three months post-hospitalization, advised by the neuroradiologist, showed no concerning radiological signs, indicating the inflammatory lesions had resolved. However, despite ongoing physiotherapy, the child's right arm still showed significant motor deficits. Electromyography (EMG) of the right arm highlighted this motor deficit, particularly in the upper right segment, though sensory parameters remained intact.

Given the comprehensive treatment and physiotherapy, the expectation is for continued, albeit gradual, recovery. However, long-term monitoring is crucial due to the unpredictable nature of myelitis and the potential for recurrences or complications.

Discussion

ATM in pediatric populations presents a unique diagnostic challenge due to its rarity, broad spectrum of clinical manifestations, and potential to lead to severe disability if not promptly treated. This case of a 3-year-old male presenting with ATM and paralysis of the right arm underscores the importance of early diagnosis, aggressive treatment, and comprehensive follow-up care.

Our case is similar to previously reported pediatric ATM cases in terms of presentation. ATM often manifests with motor, sensory, and autonomic dysfunction related to the level of the spinal cord affected.[8] While our patient presented predominantly with right arm paralysis, many patients manifest with symptoms corresponding to the affected segment of the spinal cord, including paraparesis, quadriparesis, and sensory level.

The majority of ATM cases in children are known to have a preceding trigger, most commonly a viral or bacterial infection. This post-infectious etiology suggests an immune-mediated pathogenesis, wherein an aberrant immune response following infection affects the spinal cord.[2] In our case, though the child presented with fever and elevated neutrophils, pinpointing a specific preceding infection proved challenging.

As the literature highlights, MRI plays a pivotal role in diagnosing ATM.[4,5] Our findings of inflammatory lesions in the cervical spinal region echo those of other reports, emphasizing the diagnostic significance of MRI in ATM. While typical ATM cases usually display longitudinally extensive lesions spanning multiple vertebral segments, our patient had a localized cervical involvement, making it a relatively unusual presentation.

Therapeutic management of ATM largely revolves around high-dose corticosteroids, as they have demonstrated efficacy in reducing inflammation and improving outcomes.[9] In line with this, our patient underwent high-dose Methylprednisolone therapy. However, despite the pharmacological intervention, the child exhibited persistent motor deficits in the right arm, highlighting that some patients might exhibit residual symptoms post-treatment.

The use of adjunct therapies like IgG and antiviral medications, as in our case, is driven by the underlying suspected etiology and the clinical presentation of the patient. There's evidence that therapies like IgG can modulate the immune response and provide potential benefits, especially in inflammatory conditions.[10]

Furthermore, physiotherapy plays a pivotal role in the recovery phase. Our patient's slight improvement in the

right arm mobility at the end of in-patient treatment can be attributed to the early initiation of physiotherapy. This is in line with findings from Greenberg et al.,[11] who emphasized the importance of rehabilitation in improving functional outcomes for ATM patients. There is a chance that about 5% to 10% of patients with TM will develop multiple sclerosis.[12] It is therefore important that an inter-professional team that provides a holistic and integrated approach to acute and post-acute patient care with transverse myelitis can help achieve the best possible outcomes. While similarities can be drawn from existing literature, each case adds valuable insights to the ever-evolving understanding of ATM.

Conclusion

This case report highlights the challenges in diagnosing and managing acute transverse myelitis in pediatric patients. The disorder's diverse clinical presentation, rapid progression, and potential long-term motor deficits necessitate a comprehensive and multidisciplinary approach to care.

Early recognition, prompt initiation of high-dose corticosteroids, and appropriate supportive therapy, including physiotherapy, play a crucial role in achieving favorable outcomes in children with acute transverse myelitis. However, long-term follow-up and rehabilitation are essential to address persistent neurological deficits and promote functional recovery.

Further research and case studies are needed to better understand the etiology, pathophysiology, and optimal management strategies for pediatric patients with acute transverse myelitis. The lessons learned from this case can contribute to the development of evidence-based guidelines for the diagnosis and treatment of this rare neurological condition in children.

Competing Interests

The authors declare that they have no competing interests.

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International Journal of Biomedicine 13(4) (2023) 371-373
http://dx.doi.org/10.21103/Article13(4)_CR2

CASE REPORT

INTERNATIONAL JOURNAL OF BIOMEDICINE

Pulmonary and Renal Thromboembolism Occurring within First Hours after Knee Arthroscopy: A Case Report

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Abstract

Thromboembolism presents a severe medical condition in which blood clots form within blood vessels, dislodge, and travel freely within the bloodstream, sometimes blocking blood flow to vital organs, thus causing life-threatening ischemia, tissue infarction, and end-stage organ malfunction. We present the case of a 36-year-old male patient who underwent knee arthroscopy that, within the first 12 hours post-surgery, was complicated with systemic thromboembolism impacting both his lungs and kidneys. A multidisciplinary approach is crucial to minimize the thromboembolism occurrence risk while maximizing the benefits of knee arthroscopy and promoting better patient health.**(International Journal of Biomedicine. 2023;13(4):371-373.)**

Keywords: systemic thromboembolism • coagulopathy • knee arthroscopy • CT scan • kidney infarction • dialysis

For citation: Ukimeraj MB, Ymeri L, Pasha F, Jashari A, Goga V. Pulmonary and Renal Thromboembolism Occurring within First Hours after Knee Arthroscopy: A Case Report. International Journal of Biomedicine. 2023;13(4):371-373. doi:10.21103/Article13(4)_CR2

Introduction

Thromboembolism presents a severe medical condition in which blood clots form within blood vessels, dislodge, and travel freely within the bloodstream, sometimes blocking blood flow to vital organs, thus causing life-threatening ischemia, tissue infarction, and end-stage organ malfunction.[1,2] Patients undergoing various surgeries, particularly orthopedic procedures requiring prolonged immobility, are at a considerably high risk for postoperative systemic thromboembolism.[3]

Among frequent orthopedic surgeries is knee arthroscopy, used to diagnose and treat knee injuries such as meniscal tear, and ligament and cartilage damage. Although knee arthroscopy is considered a generally safe procedure, deep vein thrombosis, followed by systemic thromboembolism in vital organs such as lungs, kidneys, and brain, is a common complication.[4] Risk assessment, patient education, and prophylactic measurements are used to reduce the incidence of thromboembolic events, morbidity, and mortality.[5]

The medical community continuously strives to further reduce the incidence of postoperative thromboembolism through refining strategies and protocols and advancing preventive measurements and surgical techniques, thus promoting the safety and success of surgical procedures.[6]

This case report discusses the relationship between a thromboembolism after knee arthroscopy and its incidence, clinical management, and prevention measurements.

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Case Presentation

A 36-year-old male patient underwent knee arthroscopy due to a medial meniscal tear and rupture of the anterior cruciate ligament. The procedure was performed early in the morning, under spinal anesthesia, and was considered successful as the patient felt well afterwards.

As the evening approached, the patient started feeling nauseous, had severe back pain, and vomited a few times. The patient's clinical state was initially considered a late side effect of spinal anesthesia, while his blood pressure and pulse remained normal (139/90 mmHg and 77 bpm). In less than 30 minutes, the patient started having severe chest pain and trouble breathing, and blood oxygenation levels dropped to 88%.

Another blood check-up was performed, and D-Dimer values appeared to be 2.1 µg/ml, which increased the suspicion of postoperative thromboembolism. A contrast-enhanced chest and abdominal computed tomography scan was immediately performed, thus revealing both pulmonary and renal thromboembolism (Figures 1-5).

In this case, pulmonary thromboembolism presents clot formation in main pulmonary arteries or veins, or embolus migration from lower body compartments, and it is a common postoperative life-threatening condition. Clots usually form on deep veins in the lower extremities or deep pelvis, usually due to prolonged patient immobility, reduced blood flow, and surgical trauma itself, thus disrupting normal homeostasis and triggering a coagulation cascade, which leads to a clot forming, which later breaks free and travels upward to vital organs. Due to the rapid obstruction of blood flow to the lungs, the patient begins to experience severe chest pain and shortness of breath, and in the worst case, this clinical condition can lead to cardiopulmonary arrest.[7]

Multiple pre- and postoperative measures are taken to prevent pulmonary thromboembolism, such as using intermittent pneumatic compression devices, wearing compressive socks, using anticoagulant therapy, early mobility of the patient, and frequent physical therapy.

After surgery, early patient monitoring is essential, such as frequently monitoring vital signs and oxygen saturation levels and recognizing early signs of chest pain or increased respiratory rate.[8] If pulmonary thromboembolism has already occurred post-operatively, as in our case, the treatment usually involves anticoagulant or thrombolytic therapy, oxygen therapy, and hemodynamic patient support. To reduce the risk and minimize the impact of postoperative pulmonary thromboembolism, it is crucial to take proper prophylactic measurements, monitor the patient closely, and treat them accurately.[9,10]

Unfortunately, our patient also experienced renal thromboembolism and infarction, concomitantly with pulmonary embolism, thus requiring an immediate patient transfer to the intensive care unit (Figures 4 and 5).

With kidney thromboembolism, blood clots block blood vessels supplying the kidneys, leading to severe impairment of renal function (BUN=73mmol/L, Cr= 6.3mg/dL). Our patient had an acute kidney injury that required immediate dialysis, which helped maintain proper electrolyte and fluid balance.

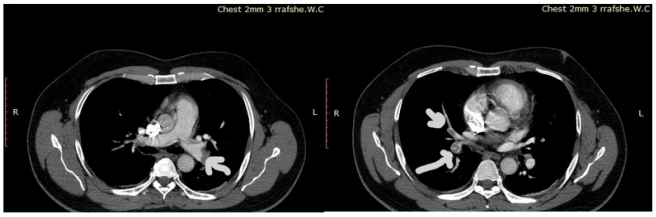

Fig. 1. *Contrast-enhanced axial chest CT scans: a. Left pulmonary artery embolism, b. Right pulmonary artery embolism (as indicated by the arrow line).*

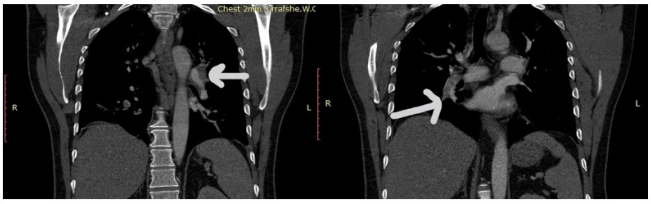

Fig. 2. *Contrast-enhanced sagittal chest CT scans: a. Left pulmonary artery embolism, b. Right pulmonary artery embolism (as indicated by the arrow line).*

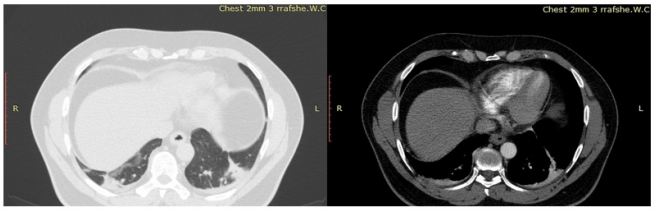

Fig. 3. *A minimal pulmonary effusion on both sides of the lungs.*

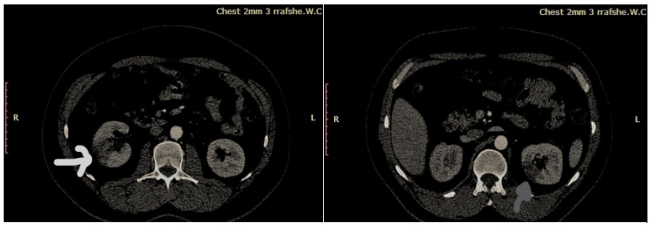

Fig. 4. *Axial abdominal CT scans: a. Right kidney infarction, b. Left kidney infarction (as indicated by the arrow line).*

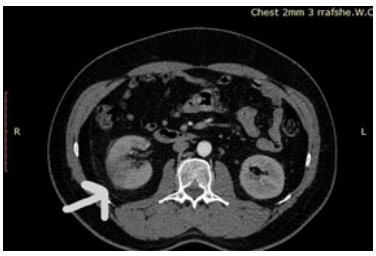

Fig. 5. *Contrast-enhanced axial abdominal CT scans: Right kidney cortical infarction*

Discussion

Various factors, such as type of surgery, prophylactic measurements, and patient's general health, define the incidence and severity of systemic thromboembolism. Overall, systemic thromboembolism is considered a very rare event after knee arthroscopy, but it can be very debilitating once it occurs.[11]

The most frequent cause of systemic thromboembolism after knee arthroscopy is the patient's prolonged immobility, leading to venous stasis and hypercoagulability, which triggers the coagulation cascade and leads to clot formation. In addition, one should consider patient-specific risk factors such as obesity, advanced age, smoking, and cardiovascular or oncologic events, which would add to this equation.[12]

To significantly reduce the risk of thromboembolism, physicians should offer proper prophylactic measurements to the patients at risk, such as the use of anticoagulant therapy such as aspirin or heparin, which can significantly reduce the risk, and make sure that patients understand the importance of their compliance with therapy, which plays a crucial role in the treatment's success.[13]

Preventing systemic thromboembolism after knee surgery is essential to ensure patient safety and optimize postoperative outcomes. Physicians should evaluate the patient's risk factors, such as advanced age, medical history, and comorbidities. Furthermore, it is critical to maintain proper hydration of the patient after surgery, educate the patient about the importance of anticoagulant compliance, and offer mechanical compression devices as integral components of a comprehensive approach to preventing systemic thromboembolism.[14]

In conclusion, a multidisciplinary approach is crucial to minimize the thromboembolism occurrence risk while maximizing the benefits of knee arthroscopy and promoting better patient health.

Competing Interests

The authors declare that they have no competing interests.

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International Journal of Biomedicine 13(4) (2023) 374-376
http://dx.doi.org/10.21103/Article13(4)_CR3

CASE REPORT

INTERNATIONAL JOURNAL OF BIOMEDICINE

Periodontal Disease as a Possible Cause of a Lung Abscess: A Case Report

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Abstract

The microbiology of a lung abscess can vary depending on the source of infection, patient risk factors, and the presence of underlying conditions. We report a case of lung abscess diagnosed in a 62-year-old female, possibly connected with periodontal disease, caused by *Pseudomonas aeruginosa* and *Porphyromonas gingivalis*, identified in the sputum. The CT scan showed a large cavitary lesion in the right lower lobe; the cavity had an air-fluid level and a smooth inner margin. The intraoral examination revealed soft and hard colored deposits, carious lesions in the molars, and two remaining gangrenous roots in the region of the upper left premolars. The periodontal examination was done based on the CPITN index, and the highest value obtained for the sextant was 3.This patient showed a remarkable improvement after a 2-month combined treatment with antibiotics therapy, combined with chlorhexidine gargle oral care, root planning, and scaling. The aspiration of contents from the oral cavity and poor oral hygiene is the leading cause of lung abscesses.**(International Journal of Biomedicine. 2023;13(4):374-376.)**

Keywords: lung abscess • oral microflora • periodontal disease

For citation: Salihu B, Salihu A. Periodontal Disease as a Possible Cause of a Lung Abscess: A Case Report. International Journal of Biomedicine. 2023;13(4):374-376. doi:10.21103/Article13(4)_CR3

Introduction

A lung abscess is described as a small region of pus or necrotic material in the lung parenchyma that causes the formation of significant cavities.[1,2] The microbiology of a lung abscess can vary depending on the source of infection, patient risk factors, and the presence of underlying conditions. The most common causative agents are anaerobic bacteria,[3] such as *Bacteroides* species (e.g., *Bacteroides fragilis*), *Prevotella* species (e.g., *Prevotella melaninogenica*), *Fusobacterium* species (e.g., *Fusobacterium nucleatum*), *Peptostreptococcus* species (e.g., *Peptostreptococcus anaerobius*). In some cases, aerobic bacteria can also contribute to lung abscess formation. These may include *Streptococcus* species (e.g., *Streptococcus pneumoniae*), *Staphylococcus aureus,* including methicillin-resistant *Staphylococcus aureus*, *Klebsiella pneumonia*, and *Haemophilus influenzae*.[3-7]

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The causative factors can enter the lungs through one of the following mechanisms:

Aspiration of Oral Bacteria: Chronic inflammation, known as periodontal disease, affects the bone and gums that support the teeth. The periodontal pockets may act as a reservoir and source of dissemination and development of systemic infections. Through the gums, bacteria can enter the circulation and can be aspirated (inhaled) into the lungs, especially in people with weakened immune systems or those who already have lung diseases.[2,8,9]

Systemic Inflammation: An inflammatory response with higher levels of inflammatory markers in the bloodstream can be brought on by chronic periodontal disease. This widespread inflammation may exacerbate current lung infections or aid lung abscess formation.[10,11]

Impaired Immune Response: Both locally in the oral cavity and systemically, periodontal disease can impair the immune system. Lung abscesses and other respiratory infections may be more likely to develop in those with weakened immune systems.[12]

Common Risk Factors: The possibility of a relationship between periodontal disease and lung abscess may be

strengthened because both conditions potentially share risk factors like smoking, diabetes, and immunosuppression.[13-15]

Case Presentation

A 62-year-old female patient reported a continuous productive cough in the previous five weeks, while in the last week, the cough has worsened with blood in the sputum. The patient was admitted to the clinic in a serious condition and had recently had a high temperature, fever, dyspnea, and chest pain, especially while lying on the right side. The patient also had bad breath, which was one of her complaints.

In the physical examination, the patient's temperature was 37.5°C, and the oxygen saturation was 93%, which was the first sign that led us to suspect pneumonia. The heart rate was 80 bpm, and the blood pressure was 95/65 mmHg. After a few minutes, we repeated the measurements and confirmed that the blood pressure was 110/70 mmHg. C-reactive protein was 10 mg/L. The laboratory tests revealed that the white blood cell count was 9.5×10^9/L (neutrophils -70.3%, lymphocytes -24.2%, and monocytes -5.5%). The CT scan showed a large cavitary lesion in the right lower lobe; the cavity had an air-fluid level and a smooth inner margin (Figure 1).

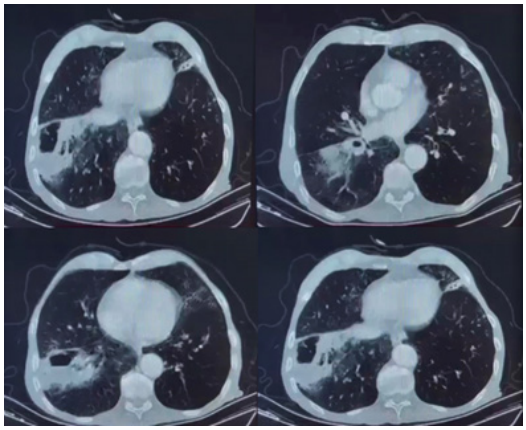

Fig. 1. *A chest CT scan on admission: A large cavitary mass on the right lower lung lobe, with slightly irregular borders.*

In the sputum obtained from the bronchoalveolar pathways, strains of *Pseudomonas aeruginosa* and *Porphyromonas gingivalis* were isolated. These findings suggested a connection with the periodontal disease present in the patient. However, a histopathological and microbiological examination of the biopsy tissue would be more adequate since contamination of the sputum from oral microbiota might occur. Knowing that the pulmonary abscess and the aspiration of the oral microbiota are closely related, after the findings in the sputum, we performed an intraoral examination. The patient reported bleeding from the gingiva during tooth brushing. The intraoral examination revealed soft and hard colored deposits, carious lesions in the molars, and two remaining gangrenous roots in the region of the upper left premolars (Figure 2). The periodontal examination was done based on the CPITN index, and the highest value obtained for the sextant was 3.

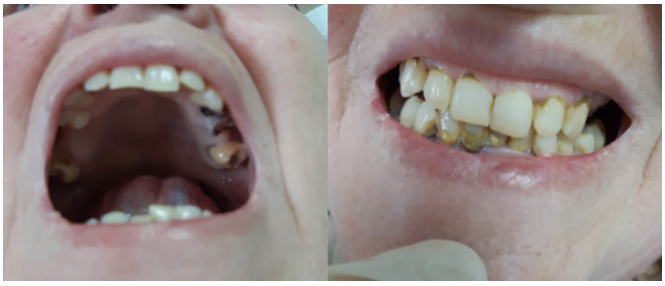

Fig. 2. *Intraoral views of the patient: The soft and hard colored deposits, mainly on the vestibular surfaces.*

Along with prescribed oral antibiotics, such as amoxicillin/clavulanate potassium (1 mg/0.5 mg, TID) and metronidazole (0.4 g, TID) for 8 weeks, the patient was also prescribed mouth rinsing with chlorhexidine and root planning and scaling with the dentist's consultation. After 8 weeks of therapy and 2 weeks of vacation, the patient's clinical and radiological signs were significantly improved (Figure 3).

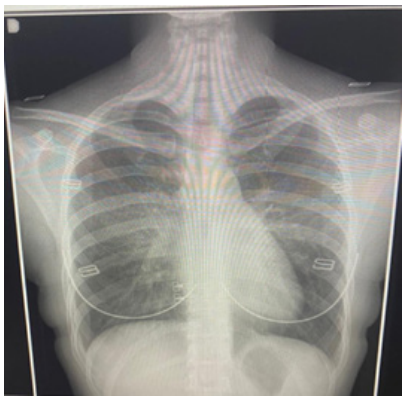

Fig. 3. *Chest X-ray 10 weeks after treatment: A reduction in the lesion size but with signs of bronchopneumonia on the same side.*

Discussion

Takayanagi et al. studied the etiological pathogens of pulmonary abscesses in Japan and concluded that periodontal disease was present in 61% of the lung-abscess patients studied.[16]

The connection between poor oral hygiene, periodontal diseases, and pulmonary abscesses was reinforced in a study by Moreira and colleagues,[17] wherein 252 cases of pulmonary abscesses were analyzed. According to the authors, 209 cases of pulmonary abscesses occurred in the men (82.9%) and 43 in the women (17.1%), whereas dental diseases were observed in 82.2%. The most important conclusion drawn from this study's results is that lung abscesses are mainly encountered in adult men who suffer from dental diseases and have a history of loss of consciousness (especially due to alcohol).

Our clinical case was like that of Guo W. et al.[9]; however, they used a newer approach with the help of biopsy, but we relied on the analysis of sputum and bronchoalveolar fluid. At the same time, if we compare our findings with those

of the aforementioned study, the suspected odontogenic agent we found is *Porphyromonas gingivalis*, a pathogen with more aggressive behavior than *Actinomyces odontolyticus*, which was found in their study. However, these pathogens isolated in different cases and by different authors suggest a possible connection between periodontal disease and pulmonary abscess.

A hundred years ago, lung abscess mortality was around 75% of affected patients. Pulmonary abscess drainage has reduced mortality by 20%-35%, and antibiotic therapy has reduced mortality by 8.7%. At the same time, it has been proven that progress in oral and dental hygiene also decreases the incidence of lung abscesses.[18] The aspiration of contents from the oral cavity and poor oral hygiene is the leading cause of lung abscesses.[19]

Competing Interests

The authors declare that they have no competing interests.

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International Journal of Biomedicine 13(4) (2023) 377-379
http://dx.doi.org/10.21103/Article13(4)_CR4

CASE REPORT

INTERNATIONAL JOURNAL OF BIOMEDICINE

Sirenomelia (Mermaid Syndrome): A Case Report

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Abstract

This report details the case of a neonate born at 39 weeks of gestation with dysmorphic features (Sirenomelia). After three hours of admission, the patient suffered from a cardiac arrest. Cardiopulmonary resuscitation was performed for 20 minutes, but there was no response, and the neonate died. Sirenomelia is an unusual and fatal congenital deformity, the most severe condition of caudal regression syndrome. This syndrome can cause pelvic-sacral dysplasia, genital anomalies, bilateral pelvic renal fusion with renal dysplasia, colon atresia, unilateral umbilical artery, and imperforated anus.**(International Journal of Biomedicine. 2023;13(4):377-379.)**

Keywords: sirenomelia • mermaid syndrome • neonate • X-ray

For citation: Al-Fakih MA, Hamd ZY, Elhussein N, Nassr SMA, Ame SA, Almortadha AA, AlArassi KI, Alorainy AI, Mustafa SA, Alhomida BA. Sirenomelia (Mermaid Syndrome): A Case Report. International Journal of Biomedicine. 2023;13(4):377-379. doi:10.21103/Article13(4)_CR4

Introduction

Sirenomelia (SML), also called the mermaid syndrome, is a rare and fatal congenital defect characterized by varying degrees of lower limb fusion, thoracolumbar spinal anomalies, sacrococcygeal agenesis, and genitourinary and anorectal atresia.(1) SML was originally described by Rocheus in 1542 and Palfyn in 1553 and named after the mythical Greek sirens.(2) The incidence of SML is 0.8 to 1 case/100,000 births, with male to female ratio being 3:1.(3) The main characteristic feature differentiating SML from caudal regression syndrome is the presence of a single umbilical artery arising at the aorta, called a "persistent vitelline artery."(4) This aberrant vessel is the basis for the hypothesis that SML results from "vascular steal" with the diversion of the blood away from the caudal embryo through the ectopic umbilical artery.(5) There are approximately 300 cases reported in the literature.(3) Except in extremely rare circumstances, SML is a fatal disease during the perinatal period, which resists any attempts at treatment.(6)

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Case Presentation

A 19-year-old primigravida arrived at the Emergency Department of Obstetrics at Palestine Hospital for Maternity and Childhood in Sana'a, Yemen. Her cervix was fully dilated and ready for vaginal delivery. During the gestational period, the mother did not seek any prenatal care and did not receive

any gestational ultrasound during pregnancy. The mother was taking supplementary folic acid that she started in the third trimester. The medical history of the mother was unremarkable, as well as the fact that the mother denied any chronic use of any medication, particularly those with known teratogenic effects. There was no family history of similar conditions, and the mother denied any consanguinity. The mother admitted to taking a herbal substance from the Qat plant, known locally as 'Al-Qatt' for recreational use during pregnancy, and she was also a smoker. The neonate was born at 39 weeks of gestation with dysmorphic features and with a gestational weight of 2.5 kg. The neonate cried immediately after birth and had an Apgar score of 5 at 1 minute and 5 at 5 minutes before becoming completely cyanosed. Resuscitation was done, intubation took place, and the neonate was shifted to the NICU. Although the patient did not resume regular spontaneous breathing, the neonate exhibited gasping breathing. As a result, intubation was performed using an endotracheal tube, and the patient was put on mechanical ventilation in synchronized intermittent mandatory ventilation mode. Following this intervention, the patient's oxygen saturation increased to 90%, and their heart rate rose to 88 bpm.

After three hours of admission, the patient suffered from a cardiac arrest. Cardiopulmonary resuscitation (CPR) was performed for 20 minutes, but there was no response, and the neonate died.

The clinical examination revealed dysmorphic features in the baby, including a wide, flat, and open anterior fontanelle, as well as a prominent flat posterior fontanelle. Additionally, during clinical examination, several other dysmorphic features were seen on the neonate, which included a wide sagittal suture, a prominent occipital bone, a depressed nasal bridge, a flat maxillary bone (displaying a 'head on chest appearance'), a short neck with folded skin, a short chest cage with widely spaced nipples, and decreased air entry bilaterally with transmitted sounds. After conducting a focused examination of each of the systems, it was noted that there were normal S1 and S2 with no added heart sounds during the cardiovascular examination. The abdomen was found to be soft and lax when the abdominal examination was conducted, and a palpable liver was detected 4 cm below the costal margin. There was a single umbilical artery and an absence of the genitalia and the anus. When examining the lower limbs, we observed a fusion of the limbs, resulting in a single limb with six digits and palpable bilateral patellae of the knees (Figures 1 and 2).

X-rays of the lower limbs displayed conjoined lower limbs with complete soft tissue fusion and partial fusion of both femora at the superior metadiaphyseal level. The two tibiae are visible, with a single fibula in the center. The feet are partially separated and exhibit a reduced number of bones (Figure 3). A skull X-ray showed a flattened occipital bone with a depressed nasal bridge. A chest X-ray showed the heart deviated to the right side with an abnormal bilateral peripheral lucency, with no definite diaphragmatic hernia (mostly related to rotation due to positioning with a suspected bilateral pneumothorax) (Figure 4). The above radiological findings are consistent with Potter syndrome type IV.[7]

Fig. 1. *Complete lower limb infusion with the absence of genitalia. A single limb with six digits and palpable bilateral patellae of the knees*

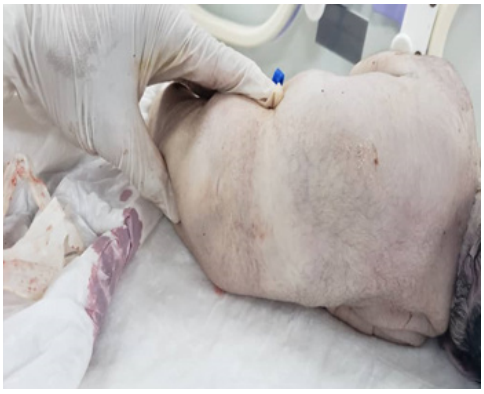

Fig. 2. *Newborn back: the absence of the anus.*

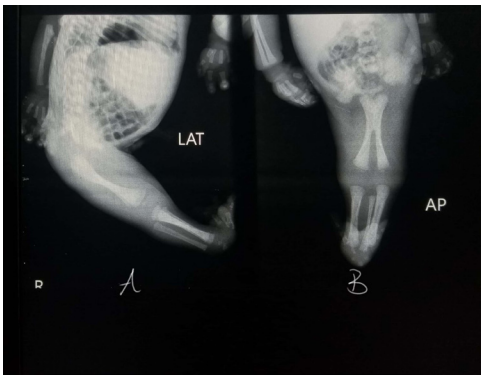

Fig. 3. *X-rays of the lower limbs: A - lateral view, B - AP view.*

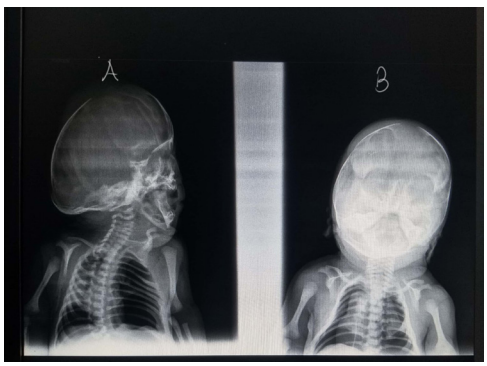

Fig. 4. *A skull & chest X-ray.*

Discussion

One of the main pathogenetic hypotheses for SML is defective blastogenesis, which is due to an abnormal development of the blastula stage during embryogenesis. Specifically, SML is characterized by a lack of mesodermal generation in the caudal region, leading to the fusion of the legs and other anomalies such as renal agenesis and gastrointestinal defects.[8]

In this case, SML was discovered after delivery due to a lack of follow-up during pregnancy. Congenital anomalies are anatomical or functional alterations that take place during fetal development. They can contribute to long-term disability, which can have serious consequences for individuals, their families, healthcare systems, and societies, in addition to being major causes of perinatal and neonatal deaths. Every year, approximately 295,000 children die during their first four weeks of life due to congenital anomalies, according to the World Health Organization.[9]

J. Serudji[10] reported, "Environmental and teratogenic factors, such as cocaine, retinoic acid, heavy metals, cyclophosphamide, and certain antibiotics, have been linked to SML in humans and animal models. In addition, nicotine, alcohol, radionuclides, diethylpropion—an appetite suppressor—organic solvents of fats, and even air pollution have been associated with SML and caudal regression syndrome, which is controversially considered as its minor form."

Additionally, Torabizadeh et al.[11] reported that maternal diabetes, genetics, irradiation exposure, and the potential teratogenic effect of vitamin A may cause SML. They also explained the association of SML with new reproductive technologies, namely with ICSI (Intra Cytoplasmic Sperm Injection).

The best method to diagnose SML is ultrasonography in the first or early second trimester. The use of transvaginal ultrasound can provide detailed information on the anatomy, while color and power Doppler ultrasound can aid in the diagnosis of a single umbilical artery. Therefore, regular ultrasound examinations are recommended to detect this congenital malformation early.[12] On the other hand, MRI is helpful in advanced cases.

SML is lethal in most cases due to pulmonary hypoplasia and renal agenesis. To prevent SML, it is important to avoid teratogenic factors and maintain normal blood glucose levels in cases of maternal diabetes. Primary prevention of SML is possible through appropriate pre-conceptional diagnosis and regular follow-up during pregnancy, especially in the first trimester.

Competing Interests

The authors declare that they have no competing interests.

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International Journal of Biomedicine 13(4) (2023) 380-384
http://dx.doi.org/10.21103/Article13(4)_CR5

CASE REPORT

INTERNATIONAL JOURNAL OF BIOMEDICINE

A Rare Case Resistant Chemotherapy Thymoma B2 Type, New Approach

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Abstract

Invasive thymomas and thymic carcinomas are relatively rare tumors, which together represent about 0.2% to 1.5% of all malignancies. The majority of thymomas (90%) are found in the anterosuperior mediastinum. Type B2 thymoma, one of the rarest cases, was diagnosed in a 35-year-old male. Anamnesis, imaging examination, serological and invasive procedures confirmed the diagnosis. In 2020, the patient was operated on with thymoma R0-resection. The patient showed no progression until July 2022, when a locoregional recurrence was detected. The patient received 6 cycles of chemotherapy, to which the tumor showed resistance. After 6 months, the patient started a treatment with tyrosine kinase inhibitor sunitinib. If a patient is diagnosed with thymoma B2 that does not respond to standard chemotherapy, the tumor can be accepted as an aggressive one requiring a change to a new treatment.**(International Journal of Biomedicine. 2023;13(4):380-384.)**

Keywords: thymoma • diagnosis • treatment • outcome

For citation: Çmega VG, Jakupi A, Agaj A. A Rare Case Resistant Chemotherapy Thymoma B2 Type, New Approach. International Journal of Biomedicine. 2023;13(4):380-384. doi:10.21103/Article13(4)_CR5

Introduction

Thymomas originate from the thymic epithelial cells within the anterior mediastinum but can be found in the posterior and middle mediastinum or neck.[1] Thymomas are associated with an exuberant lymphoid component composed of immature cortical thymocytes. Different thymoma types exhibit different patterns of thymopoiesis, and type B thymomas produce more lymphocytes than type A.[2] Thymomas are exceedingly rare. The Surveillance, Epidemiology, and End Results (SEER) Program showed a thymoma incidence of 0.15 per 100,000 person-years. The incidence was higher in males than females (P=0.007).[3] Thymomas are generally characterized by an indolent growth pattern that can be locally invasive. Thymomas are intimately associated with a wide spectrum of immunological diseases. Type B thymomas are most often associated with immune disorders. Myasthenia gravis is the most common immune-mediated disease associated with thymoma, and 30%-40% of thymoma patients suffer from myasthenia gravis.[4,5]

Case Presentation

In July 2020, a 35-year-old male patient felt symptoms of cough, chest pain, and dyspnea. After many laboratory and radiological examinations (Fig.1), the patient was found to have a mediastinal mass in the anterior mediastinum.

Later, the surgery was performed with total resection of the mass described in the pathohistological report as R0 resected margins type B2 thymoma. At that time, the patient did not receive any treatment. After 2 years, in June 2022, the patient was given another chest examination where the locoregional recurrence had been detected, along with enlarged lymph nodes in the neck (Fig.2). A second biopsy was done, and it resulted in the same pathohistological diagnosis: Thymoma B2 type (cortical thymoma), an invasive malignant subtype. The therapy included 6 cycles of chemotherapy (carboplatin, doxorubicin, cyclophosphamide), starting on 07/29/2022, with the last cycle on 11/15/2022. On CT examinations performed on 11/17/2022, the findings were compared to the examinations before treatment: Left para-aortic mass with dimensions of 1.4 cm without changes in dimensions, the subpleural mass at the 8th posterolateral rib (3.6×2.0 cm in the previous examination with current dimensions of 3.2×1.8 cm), left subpleural infiltration 3.7×1.7 cm in the last examination with current dimensions of 3.4×2.1cm. Infiltrative left paravertebral mass of 1.3×4.0 cm versus 1.8×4.5 cm before treatment. Left posterobasal pleural thickening. No metastases have been seen in the abdomen during all those years.

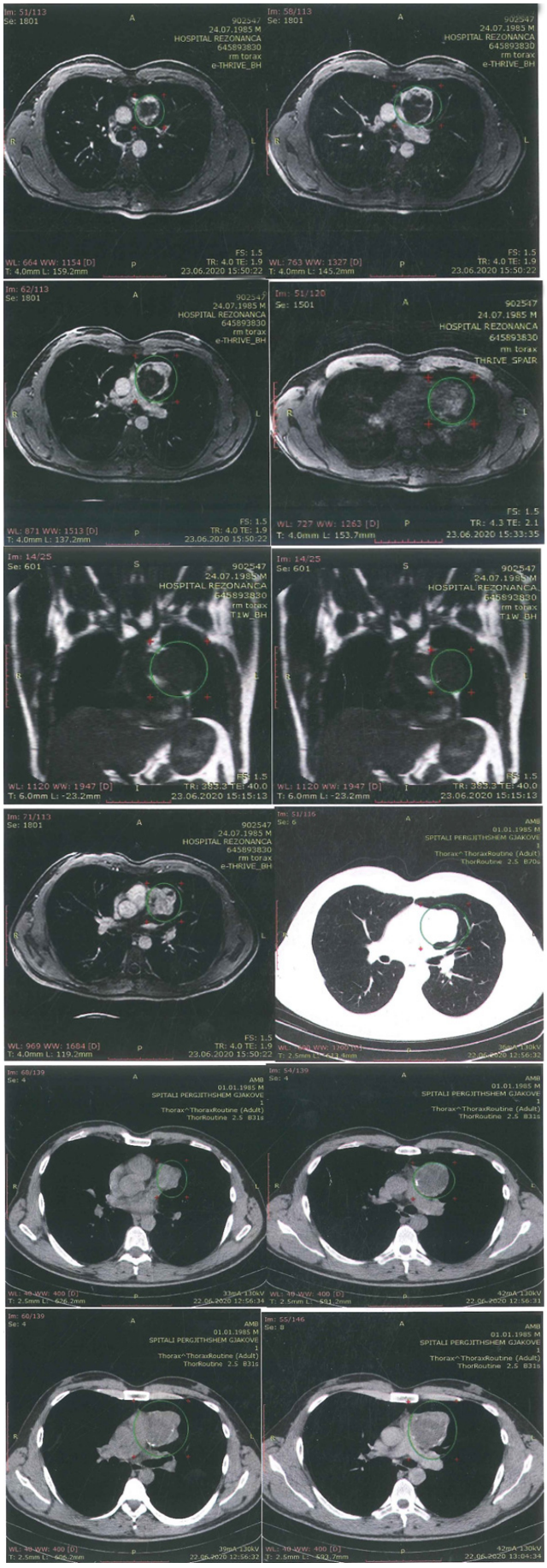

Fig. 1. *June 2020: Thorax MRI and CT before operation.*

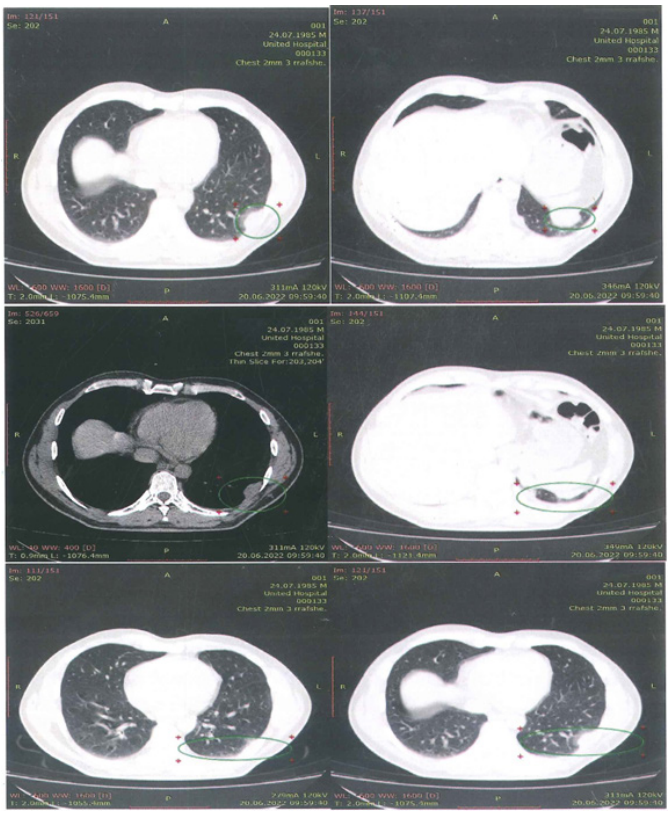

Fig. 2. *June 2022: Thorax CT before operation.*

Echocardiography (09/19/2022): LVEF-70%. Data from a large panel of tumor markers, as well as laboratory and biochemistry analysis, were in normal values before and after chemotherapy. In the biochemistry analysis on 05/09/2023, cholesterol was 7.4 mmol/l, and triglycerides - 2.69 mmol/l.

Chest CT on 05/22/2023: minimal left interlobar pleural effusion, contrast-enhancing solid mass about 70×30mm adhering to the thoracic wall in the mediastinum. Progression was detected in the radiological evaluation, performed in December 2022 (Figure 3).

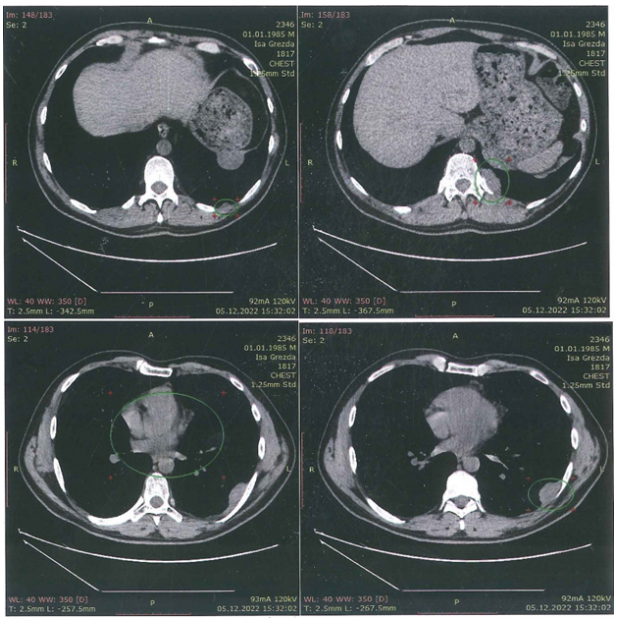

Fig. 3. *December 2022: Thorax CT after operation.*

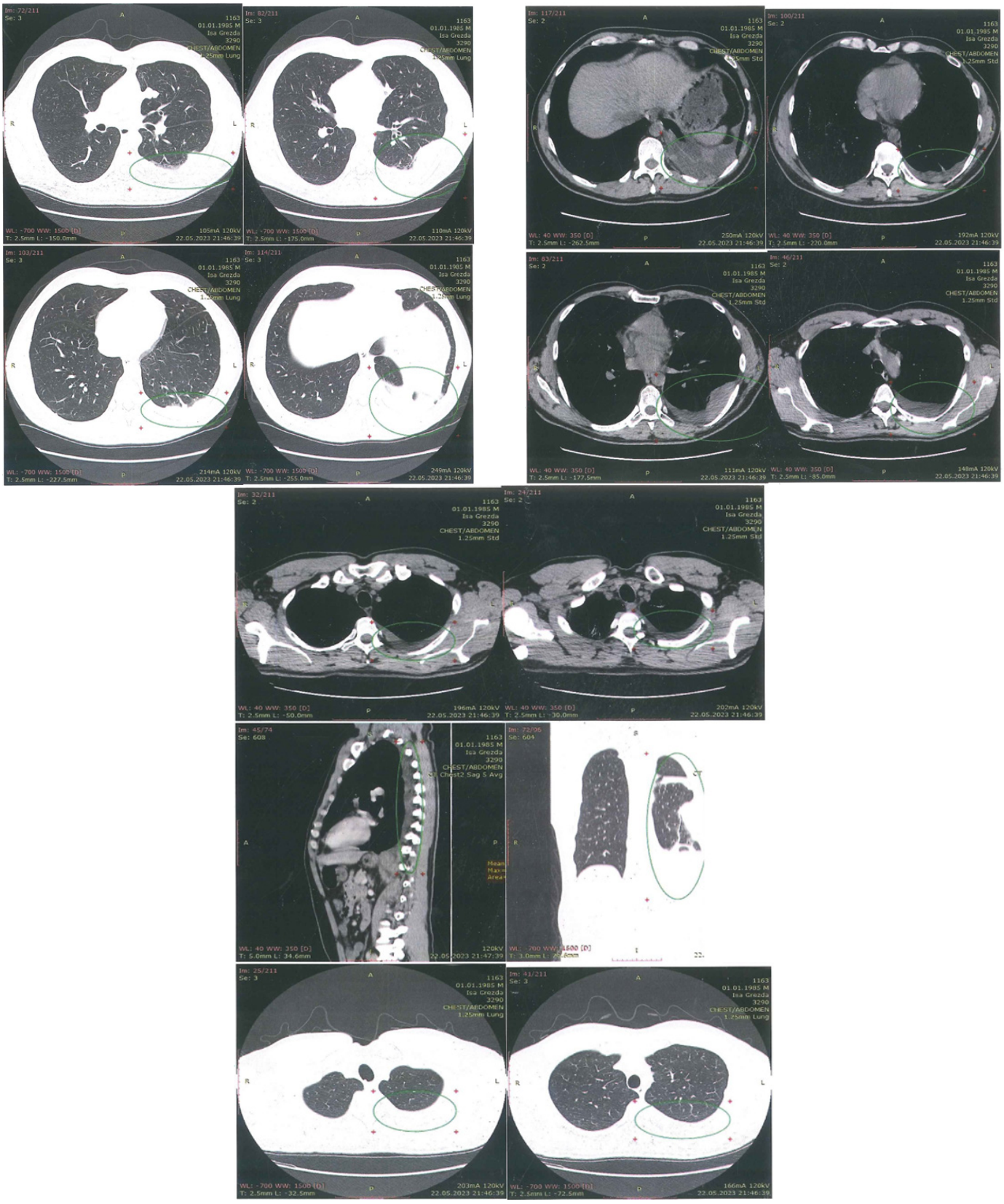

Fig. 4. *May 2023: Thorax CT after chemotherapy.*

Afterward, the patient was not treated with any kind of specific oncological therapy. On the patient's chest CT (May 2023), prominent pleural effusion and pleural mass were detected on his left side (Figure 4). The patient had no accompanying paraneoplastic syndrome findings and has started with sunitinib (Sutent) (50 mg daily for 28 days, followed by a 14-day break; this is 1 cycle of treatment). The patient was recommended to do a PD-L 1 testing, considering therapy with pembrolizumab added to carboplatin and paclitaxel for future treatment, because the patient did not have any autoimmune findings.

Discussion

The vast majority of thymomas are cytologically bland tumors, and approximately half of them are noninvasive.[6-10] Roughly one-third are asymptomatic and found incidentally on a chest X-ray.[11,12] Of those with symptoms, 40% have symptoms relating to impingement by the intrathoracic mass, ranging from cough, chest pain, dyspnea, hoarseness, super vena cava obstruction, and even tumor hemorrhage.[13] Another 30% of those with symptoms have systemic signs, and the remainder present with signs of myasthenia gravis. Most thymomas are indolent, but if the tumors spread, they most commonly implant regionally on the pleural surfaces and can cause pleural plaques, diaphragmatic masses, and malignant pleural effusion. Thymomas have been extensively studied by pathologists. Many different classification systems have been proposed and used. The major distinction has been described as noninvasive thymomas versus invasive or, alternately, benign versus malignant. Even bland-appearing, noninvasive thymomas have fundamental characteristics of a malignant tumor in the ability to recur and metastasize.

According to the WHO classification, thymic epithelial tumors are classified into thymomas (types A, AB, B1, B2, and B3) and thymic carcinomas, based on the morphology of epithelial cells and the ratio of lymphocyte-to-epithelial cells.[14] Type B2 thymoma is characterized by increased numbers of single or clustered polygonal or dendritic epithelial cells intermingled with abundant immature T cells. Tumor cells tend to palisade around blood vessels and fibrous septa. Enlarged perivascular spaces are often found.[15]

Among factors that affect the prognosis and treatment options of thymomas, the invasiveness and completeness of resection should be highlighted.[6,7] A large multi-institutional survey from Japan reported 5-year survival rates in 1,320 patients of 100%, 98.4%, 88.7%, 70.6%, and 52.8% for Masaoka stages I, II, III, IVa, and IVb, respectively.[16] In a study by Cowen et al.,[17] 10-year disease-free survival rates were 92%, 87%, 60%, and 35% for stages 1, II, Ill, and IV, respectively. In a study by Regnard et al.,[18] 15-year disease-free survival rates for stages I, Il, II, and IV were 78%, 73%, 30%, and 8%, respectively.

The extent of resection is the other major prognostic factor. Patients with an R0 resection have significantly improved survival over those with R1 or R2 resections. While an R0 resection is almost always accomplished in stage I tumors, resectability rates decrease on average to 50% in stage III tumors.

Some studies have suggested that tumor size also may be a prognostic factor in thymoma.[19] Bian et al.[20] analyzed the SEER database and found that tumor size was associated with postoperative disease-specific survival and overall survival. Fukui et al.[21] found that tumor size >4cm was an independent prognostic factor for recurrence-free survival. In a study by Okumura et al.,[22] the 10-year recurrence-free survival rate was 93.8% in patients with a tumor ≤5.0 cm and 84.3% in patients with a tumor >5.0 cm (P<0.0001).

Age also has been suggested as a prognostic factor. Patients younger than 30 to 40 have a better prognosis.[17,23]

General management

Surgical resection is the mainstay of treatment for thymomas. A complete block surgical resection (RO) remains the treatment of choice for all thymomas regardless of invasiveness, except in rare, advanced cases with extensive intrathoracic or extrathoracic metastasis. Fortunately, the vast majority (90% to 95%) of thymomas are localized. Operative mortality averages 2.5% (0.7% to 4.9%).[18,24-27]

Because the completeness of resection is such an important prognostic factor, an aggressive surgical approach is justified to remove as much of the lesion as possible at the time of surgery. R1 represents a patient who has undergone tumor resection but still has residual microscopic disease present.

Although with total surgical resection, a five-year survival rate of 60% has been reported, the long-term prognosis is poor and depends on the progression of the disease and the degree of tumor differentiation.

The overwhelming recurrence pattern for thymomas is locoregional. Eighty-one percent of recurrences are local, 9% distant, and 11% both.[18,25-28]) Most recurrences arise within 3 to 7 years.[18,24,27,28] Maggi et al.[24] reported clinical and histopathological aspects of 241 thymomas that were reviewed. Radical resection was performed in 87.5% of the patients, subtotal resection with residual tumor in 8.7%, and simple biopsy in 3.7%. A tumor relapse was observed in 24(10%) patients: 2(1.5%) of 133 with encapsulated thymomas and 22(20.4%) of 108 with invasive thymomas.

Thymomas have proven to be very sensitive to chemotherapy. A clinical response is seen in roughly two-thirds of patients. Complete responses are seen about a third of the time. The commonly employed drugs in combination chemotherapy are cisplatin, doxorubicin, and cyclophosphamide. One prospective intergroup study reported disappointing results with combined etoposide, ifosfamide, and cisplatin.

Targeted drugs such as tyrosine kinase inhibitors and mammalian target of rapamycin (mTOR) inhibitors are targeted therapies used in the treatment of thymoma and thymic carcinoma. Tyrosine kinase inhibitors block signals needed for tumors to grow. mTOR inhibitors block a protein called mTOR, which may keep cancer cells from growing and prevent the growth of new blood vessels that tumors need to grow. To treat recurrent thymoma or recurrent thymic carcinoma, tyrosine kinase inhibitors sunitinib or lenvatinib and mTOR inhibitor everolimus may be used.

In conclusion, for thymoma management, a multimodal approach is recommended, including surgical resection, postoperative radiation, chemotherapy, and target therapy. Specific targeted therapy for treating thymic malignancies has shown promising results in some small clinical studies. The prognosis of thymic carcinoma is poor due to the early involvement of the lymph nodes pleura, lungs, mediastinum, cervical and axillary lymph nodes, brain, bone, and liver metastasis. Median survival and disease-free survival are very encouraging when molecular therapy is integrated into cytostatic treatment. Type B2 thymoma, known as cortical or polygonal cell thymoma, can be considered an invasive malignant tumor.

Competing Interests

The authors declare that they have no competing interests.

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