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FETAL AND NEONATAL NEUROLOGY

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COVID-19 Infection

Guest Editorial

INTERNATIONAL JOURNAL OF BIOMEDICINE

SARS-CoV-2 Biochemistry, Transmission, Clinical Manifestations, and Prevention

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Abstract

The first human case of COVID-19, caused by the novel coronavirus, was reported by health officials in the city of Wuhan, China, in December of 2019. The virus was identified as a novel coronavirus in early January 2020, and its genetic sequence was shared publicly on January 11, 2020. The novel virus, previously called 2019-novel coronavirus (2019-nCoV), is currently designated as the severe respiratory syndrome coronavirus-2 (SARS-CoV-2). On January 23, Wuhan was locked down, and the World Health Organization (WHO) declared a "public health emergency of international concern." The viral genome of SARS-CoV-2 is around 29.8 kilobase, containing six major open reading frames. The most common clinical symptoms were fever, cough, fatigue, shortness of breath, dyspnea, muscle ache, headache, chest pain, vomiting, sore throat, and sputum production. The main mode of transmission is through respiratory particles. The incubation period is 3 to 7 days. Both asymptomatic and symptomatic patients seem to be infectious. Spike (S) proteins of SARS-CoV-2 seem to have a 10- to 20-fold higher affinity to the human angiotensin enzyme 2 (ACE2) receptor than that of SARS-CoV. The high affinity of S protein to theACE2 receptor, and the additional advantages offered by the transfection facilitators Furin and Neutropilin-1, likely, contributes to the rapid spreading of this novel virus. Since these receptors are highly expressed on a variety of cells, including vascular endothelial cells and adipose tissue, individuals with compromised function of these tissues drive greater infection and severity in patients with COVID-19. Global health experts estimate that one in five individuals worldwide could be at risk for severe COVID-19, due to underlying health conditions. There is a great need for a rapid, specific, cost-effective test for monitoring the infected individuals. Even though a 15- minute, antigen test was made available by Abbott recently, it seems that the schools, colleges, and business establishments lack the ability to use these tests effectively to keep their businesses open safely. Management of the infected individuals seems to be based on clinical symptoms that manifest as the disease progresses. The US Food & Drug Administration (FDA), has created a special emergency program for possible therapies, the Coronavirus Treatment Acceleration Program (CTAP). The program uses every available method to move new and emerging treatments as quickly as possible, keeping in mind the safety and efficacy of such therapies. According to the WHO report, there are currently more than 150 COVID-19 vaccine candidates under development. Several vaccines are in Phase 3 clinical trials. In an unprecedented effort, one of the experimental monoclonal antibody cocktails of Regeneron was used for therapeutic purposes when the US president was tested positive for COVID-19. There are no drugs or other therapeutics approved by the US FDA to prevent or treat COVID-19. The National Institutes of Health (NIH) have published interim guidelines for the medical management of COVID-19. In the absence of a cure, the only choice we all have is to follow the best practices recommended by the public health experts—use of face masks (coverings), frequent hand washing with soap, contact tracing of infected individuals, and quarantining COVID-19 positive individuals, till they are free of the highly infectious virus. (**International Journal of Biomedicine. 2020;10(4):303-311.**)

Key Words: COVID-19 • clinical trial • vaccine • public health

Introduction

SARS-CoV-2, the most potent killer virus, has caused an unprecedented pandemic worldwide. It has rapidly spread, and as of today (November 2, 2020), the virus has infected more than 44 million individuals and caused 1.2 million deaths. The USA (9.24 M), India (8.29 M), and Brazil (5.5 M) are the top three countries, with the highest number of COVID-19 positive cases (coronavirus.jhu.edu/map.html). Science magazine (ScienceMag.org) has been publishing titles of current articles, which serve as a great source of information on the Latest Coronavirus Research.

A recent Comment (September 26, 2020), published in the journal Lancet, says that COVID-19 is not a pandemic—it is a syndemic. Syndemics involve the clustering of two or more diseases within a population; the biological, social, and psychological interaction of those diseases; and the large-scale social forces that precipitate disease clustering in the first place.[1] According to the experts, a syndemic approach reveals biological and social interactions, that are important for prognosis, treatment, and health policy. Therefore, limiting the harm caused by SARS-CoV-2 will demand far greater attention, to Noncommunicable Diseases (NCDs), and socioeconomic inequality, than has hitherto admitted. Disparities that exist in the rate of infection and severity of this disease in the African American and minority communities, substantiate this observation by the experts. For just argument sake, COVID-19, for instance, is a pandemic over another pandemic— cardiometabolic disease. If we were to stretch our imagination further, we will have to include all the metabolic disease risk factors also as co-existing conditions. Addressing COVID-19 management, therefore, means, addressing all the metabolic risk factors as well as metabolic diseases such as; hypertension, excess weight, endothelial dysfunction, inflammation, obesity, diabetes, vascular diseases, and chronic respiratory diseases. SARS-CoV-2 pandemic is a public health workers nightmare, as well as that of critical care workers.[2-8]

The "Spanish" influenza pandemic of 1918-1919—the mother of all the pandemics, which infected more than a quarter of the global population at that time, and caused 50 million deaths worldwide, came in several successive waves. [9] According to the researchers of Armed Forces Institute of Pathology, Bethesda, Maryland, 75 years of research failed to answer the most basic question about the 1918 pandemic: why was it so fatal? Did some critical viral genetic event produce a 1918 virus of remarkable pathogenicity? After over a century, we have been confronted with the same question again, about the pathogenicity and the severity of SARS-CoV-2, which has devastated the global health and economies. The first pandemic influenza wave appeared in the spring of 1918, followed in rapid succession by much more fatal second and third waves, in the fall and winter of 1918-19, respectively. Dr. Michael Osterholm, the director for the Center of Infectious Disease Research and Policy at the University of Minnesota, now says, -the wave model doesn't seem to apply to this disease, "I don't see we're going to see one, two and three waves – I think we're just going to see one very difficult forest of fire cases." The epidemic is now entering a more serious and complex period. In just three days, globally, over one million COVID-19 positive cases were reported. In the US, currently, we are seeing more than 90,000 COVID-19 test positive cases per day and it is predicted to exceed 100,000 a day soon. The estimated cost of the COVID-19 pandemic is more than $16 trillion, or approximately 90% of the gross domestic product of the US.[10]

Evidently, the great unmet need of the day worldwide—is the availability of a safe, rapid, specific, and inexpensive test for population screening for the virus. During a pandemic, the first thing that the public health workers need is the ability to track and manage the spread of the pathogen. Since SARS-CoV-2 was a novel virus, new tests had to be developed for testing. China developed its own test. The WHO adapted a test that was developed in Germany. The WHO supplied tests for over 150 countries. The Center for Disease Control (CDC), decided to develop its own test, as is the usual case in the USA. Jeremy Konyndyk, a senior policy fellow at the Center for Global Development explained, "That's how we normally do things. A lot of countries don't have the capabilities that we have here. And therefore, the need to rely on WHO, to provide tests to them. We don't have to do that in the United States." According to The New York Times, Health and Human Services (HHS) Secretary, Mr. Alex Azar was 'unable' to get either CDC or the FDA to 'speed up or change course.' "Moreover, he had been at odds for months with the 'White House' over other related issues." Experts put the failure simply: "The reason for the lack of sufficient testing has been such a damaging shortcoming in the U.S. response, it has basically left us blind to the spread of the virus in our country for six or seven weeks." Without enough testing, the response will continue to fall short. Now the public health workers as well as the White House Coronavirus Task Force have given up the idea of contact tracing entirely.

Because of the many types of tests available worldwide, there is considerable confusion regarding the sensitivity and efficacy of these tests. None of the tests detect the live virus, which must be confirmed only by culturing the virus. There are currently two types of tests: molecular and antigen testing. Molecular tests include; PCR test, viral RNA test, and nucleic acid tests. These tests look for genetic material that comes only from the virus. A molecular test using a deep nasal swab is usually the best option because it will have fewer false negative results. The other test is the antigen test, which detects protein fragments (antigens) from the virus. The reported rate of false negative results is a high as 50%, which is why the antigen tests are not favored by the FDA, as a single test for monitoring active infection. Abbott's rapid point-of-care test achieved a sensitivity of 93.3% and specificity of 98.4% in 1,003 subjects in a post-authorization study. Abbott's fast, 15-minute, easy-to-use COVID-19 antigen test, and received FDA emergency use authorization. The mobile App provided by Abbott displays test results, to help develop protocols for a safe return of individuals to working places, such as schools, colleges, and various business establishments. However, the reality is far from the claims made by the manufacturers and promoters. Despite the availability of these tests, schools, colleges, and businesses have not been able to accomplish testing, tracking of positive cases, and managing the overall spread of the infections. Therefore, the efforts of schools, colleges, restaurants, and various other business centers have failed, to achieve the goals of a safe opening of the working places. Brandsma and associates have reported a new, rapid, sensitive, and specific assay using CRISPR technology, which seems to be superior to the qRT-PCR method.[11]

To add to the confusion of the testing fiasco, in a recent article in JAMA (October 8, 2020), Andy Slavitt a Distinguished Fellow at the Leonard Davis Institute of Health Economics, University of Pennsylvania, reflects on the global pandemic and questions about what kind of science, the

society is not effectively using.[12] He goes further to say, "that the greatest unmet need in successfully fighting coronavirus disease 2019 in the US relates, to the insights provided by the social sciences, rather than by the traditional biomedical sciences. Sociologists and Psychologists are as important in this crisis as virologists and epidemiologists." We partially agree with the fact that in an unprecedented health crisis, sociologists and psychologists are needed to help the affected individuals and their families. Having said that, we would like to inform the readers, of the great contributions of biomedical scientists, cellular and molecular biologists, biotechnologists, epidemiologists, emergency medicine staff, clinicians, and critical care workers. The fact that a reputed journal like the Journal of American Medical Association published this article, indicates the confusion that exists in COVID-literature. No one is disputing the role of other associated sciences in the management of the COVID-19 pandemic and its impact on the global population. But the role of traditional biomedical sciences cannot be ignored.

Next to rodents, bats form the second largest mammalian species in existence and are found just about everywhere. A 2017 study of 12, 333 bats, found that almost 9% carried at least one of 91 distinct coronaviruses, and there are at least 3200 coronaviruses that infect bats. They are the natural hosts, for a whole host of known viruses such as Rabies, Ebola, SARS-CoV, HKU1, and OC43, which cause the common cold.[12] Jay Cohen reported as early as in January of 2020, in Science journal, that Chinese researchers have characterized a draft genome of the virus implicated in the Wuhan outbreak.[14] According to the Center for Infectious Disease Research and Policy (CIDRAP Jan 11, 2020), in quickly moving developments, health officials from Wuhan, China, posted a novel outbreak of coronavirus, as well as released the genetic sequence of the 2019-nCoV.[15] Based on this information, the same day, the WHO released several interim guidance documents, including travel advice, the need for lab testing, and medical evaluation. Researchers from the IBM Watson Research Center, New York, published details of their findings on variant analysis of SARS-CoV-2 in May of 2020 in the WHO Bulletin.[16] The virus showed a high nucleotide sequence homology, with two severe acute respiratory syndrome viruses—SL-CoVZC45 (88%) and with SARS-CoV (80%). SARS-CoV-2 is a coronavirus with a 29, 903-base pair (bp), single-stranded RNA genome, containing 14 open reading frames and 27 estimated proteins. The Consortium of Global Initiative on Sharing All Influenza Data (GISAID) classifies this virus into 2 main clades based on their origin: (1) clade 19A originating from China, and (2) clade 20A originating from Europe. Phylogenic analyses of the Los Angeles isolate, with genomes from New York, Washington-State, and China, found that they shared similarities to all subclades, derived from these regional locations.

According to the early reports, the virus causes the coronavirus disease 2019 (COVID-19), with common symptoms such as fever, cough, shortness of breath, and fatigue. Early data indicated, that about 20% of patients who developed severe COVID-19, required hospitalization, including 5% who needed intensive care. Initial estimates of the case fatality rate (CFR) were from 3.4% to 6.6%. The older the patients, the more severe the illness. In the early reports from China, the median age of patients was 51 years. They did not report any significant difference in age distribution between male and female patients. The overall CFR was 5.6%, which was significantly lower among female patients. This was true across the United States, as well. The coronavirus is killing more men than women. The mortality from COVID-19 was higher in people older than 65 years and in people with underlying comorbidities, such as chronic lung disease, serious heart conditions, high blood pressure, excess weight, obesity, and diabetes. Global health experts estimated that 1.7 billion people of the global population have at least one underlying condition that puts them at increased risk for severe coronavirus disease. Do your genes predispose you to Covid-19? Blood types bear two kinds of saccharide molecules on the surface of red blood cells; A and B, -and each kind of molecule is produced by an enzyme, whose enzyme exists in two forms (A & B). A third gene variant encodes an inactive enzyme: type O. Each sugar A or B may act as an antigen. It can trigger antibodies that target the antigens it lacks. Type O blood is the best for antibody production, processing both anti-A and anti-B, and therefore offers the most protection. As to the mode of transmission, the virus is mostly transmitted through the respiratory tract. The infection occurs in the respiratory tract, and emissions from the tract are the propulsion mechanism in the environment and towards others. It is therefore important to understand, that not only the distance (social distancing), but also the concentration of the viral load, and of course, the time scales of exposures. The virus is never emitted in the air on its own. It is always in the mucosalivary secretion that is emitted from either breathing out, talking, coughing, or sneezing.

The SARS-CoV-2 global outbreak is one of a kind syndemic that occurs once in a century. Since this potent killer virus is new, and novel, not much of its mode of transmission, pathophysiology, clinical symptoms, and management was known in the early days of its outbreak. In view of this fact, public health experts, emergency medicine workers, clinicians, and critical care workers were scrambling for information. During the days that followed the initial discovery of this virus, there was a great rush for publication of articles on this topic. In a recent article in Science, Jeffrey Brainard reports, on how researchers face hurdles to evaluate, synthesize COVID-19 evidence at top speed.[17] The team analyzed more than 35,000 papers and reprints about COVID-19 in a database called Epistemonikos. Some of the revelations are unbelievable. For example, an unpublished analysis of some 240 reviews, about drug treatments for the disease found, 95% were already out of date. According to these investigators, a high percentage of COVID-19 reviews were found, to be incomplete or irrelevant only months after publication, and this observation is unprecedented, and demonstrates the complexities of this pandemic. They also found that 53 reviews, dealt with the effectiveness of hydroxychloroquine, an antimalarial drug, which has been used to treat COVID-19. Although President Donald Trump and other political leaders have touted it as a remedy, the reviews have not found any evidence, for its

benefits so far. Confusions in the management of COVID-19 becomes quite evident when you just analyze the number of drugs that were administered (VIP Syndrome?) to the President of the USA when he contracted the COVID-19. The drugs included infusion of 8.4 grams of Regeneron monoclonal antibody cocktail, Remdesivir, Dexamethasone, Zinc, Vitamin D, Aspirin, Famotidine, and Melatonin.

Discussion

A new virus-associated disease was initially reported in China on 30th December 2019.[18] Early researchers generated virus genome sequences from 53 patients in Guangdong, China, using both metagenomic sequencing and multiplex PCR amplification, followed by nanopore sequencing.[19] Many countries are now investing efforts in genomic surveillance of SARS-CoV-2, and the GISAID public database has now reached, 25,995 full genomes at unprecedented speed.[20] "The U.S. is the World's leader in advanced rapid genome sequencing," This coordinated effort across our public, private, clinical, and academic public health laboratories, will play a vital role in understanding the transmission, evolution, and treatment of SARS-CoV-2," said US CDC Director, Robert Redfield. Researchers at the National Institutes of Health (NIH), USA, developed approaches, combining advanced machine learning methods, with well-established genome comparison techniques, to identify potential genomic determinants of pathogenicity of the high-CFR coronavirus strains.[21] The hypothesis being, -that the high-CFR virus strains are more pathogenic due to shared genomic determinants, that are absent in the low-CFR strains. These researchers found that within the nucleocapsid, which predicts high CFR for this virus, there were deletions and insertions resulting in a substantial enhancement of the motifs that determine nuclear localization, specifically, in high-CFR coronavirus. Genomewide Association (GA) Study of Severe COVID-19 revealed that the risk allele GA of rs11385942 was associated with reduced expression of CXCR6 and increased expression of SLC6A20, and LZTFL1 was strongly expressed in human lung cells. They also found that the frequency of the risk allele of the lead variant 3P21.31 was higher among patients, who received mechanical ventilation, than among those who received oxygen supplementation.[22]

Coronaviruses have positive-sense RNA genomes, consisting of six conserved proteins. The conserved proteins are the polyproteins pp1a and pp1b, which encompass multiple protein domains involved in various aspects of coronavirus genome replication, spike protein (S), envelope (E), membrane glycoprotein (M), and nucleocapsid phosphoprotein (N). The size of this virus is between 60 nanometers (nm) to a maximum of 140 nm. Respiratory droplets are typically 5-10 micrometers and each droplet may contain 250 virions, which means just normal talking can generate more than 750,000 virions. According to the experts, the infectious dose of SARS-CoV-2 is probably like SARS-CoV, approximately 300 virions. Masks have been recommended primarily, to reduce SARS-CoV-2 transmission, rather than reduce the dose of infectious particles. In the absence of reliable data from randomized studies, there seems to be lots of confusion, about the efficacy of masks in preventing transmission of this virus. It is encouraging, that data have emerged from the state of California, for instance, to change its public health messaging; "masks protect you and others." The Institute for Health Metrics and Evaluation (IHME), University of Washington, currently projects, that more than 360,000 Americans will die by the end of 2020. It does not have to be this way. Promoting the use of masks, social distancing, and contact tracing could still save 100,000 lives—saving lives does matter. Russian President Vladimir Putin has taken his most drastic measures yet, to curb the second wave of COVID-19. He ordered on Tuesday (October 27, 2020) a nationwide mask mandate (which becomes effective this week), as coronavirus cases spike worldwide. The country has the fourth-highest number of COVID-19 cases in the world, behind the US, India, and Brazil. The other three countries have not ordered a countrywide mask mandate.

In the high-CFR strain, the nucleocapsid protein and the spike protein were significantly enriched. The N protein is multifunctional, contributes to viral transcription efficiency and pathogenicity. The SARS-CoV-2 spike proteins bind the ACE2 host receptor with a 10 to 20-fold affinity compared to SARS-CoV and contain a polybasic furin cleavage site, resulting in a unique insert to SARS-CoV-2 that enhances infectivity. Cleavage of S generates a polybasic Arg-Arg-Ala-Arg C-terminal sequence on S1 and S2. Furthermore, their analysis revealed a four-amino acid insertion in the long connecting region between the fusion peptide of the spike protein, in all high-CFR viruses, but not in low-CFR ones. Yet another difference they noticed was, increased positive charge of the amino acids, comprising the Nuclear Localization Signals (NLSs), a known marker of NLS strength (20). Recent findings suggest the interaction of yet another receptor, called neruopilin-1(NRP1), and neuropilin 2 (NRP2) that facilitate the entry of this virus into cells. Neuropilin-1, known to bind furin-cleaved substrates, "significantly potentiates SARS-CoV-2 infectivity, and is blocked by a monoclonal blocking antibody against NRP1." Despite this knowledge, about the molecular rearrangements that modulate the transmission, pathogenicity, and severity of the coronavirus disease, little is known about the reasons for the disproportionality in the infectivity and case fatality rates among the countries across the world.

The spike protein is a type 1 transmembrane protein, comprising 1255 amino acids and seems to be the key to the host cell interactions. The virus has undergone significant mutations as it evolved worldwide. However, S Protein seems to be the key determinant of evolution, transmission, and virulence of SARS-CoV-2.[23] Coronavirus entry into host cells is mediated, by the transmembrane spike (S) glycoprotein, that forms homotrimers protruding from the viral surface.[24] This protein comprises two functional subunits, responsible for binding to the host cell receptor (S1subunit), and fusion of the viral and cellular membranes (S2 subunit). For all viruses of this group, S unit is further cleaved by host proteases, at the S2 site of the fusion peptide. Because of this mode of transmission, coronavirus entry into the host cell is a complex

process, that requires both receptor binding and proteolytic processing of the S protein, to promote virus-cell fusion.[25] Chinese researchers studied the variations in SARS-CoV-2 spike protein cell epitopes and glycosylation profiles during global transmission course of COVID-19 and concluded, "Our research offers a novel perspective on the distribution characteristics of the relatively high frequency of amino acid variations, the impacts of T and B cell epitope variants, and the conserved glycosylation sites of SARS-CoV-2 S-protein, during global transmission. The SARS-CoV-2 *S* gene encodes 22 N-linked glycan sequons per promoter, which likely play a role in protein folding and immune evasion. This knowledge will contribute significantly to the evaluation of the vaccine candidate immunogenicity, as well as monitoring of the potential consequences of glycosylation and cell epitope variations, in the process of viral transmission.[26]

SARS-CoV-2 entry into a cell involves the interaction of its spike protein with the cell's membrane-bound angiotensin-converting enzyme 2 (ACE2) which is cleaved by the transmembrane protease serine 2 (TMPRSS2), suggesting that co-expression of both genes is required for infection.[27] According to experts, there are four important enzymes that are essential for the pathogenesis; the S-protein that facilitates virus entry through the ACE2 to the host cell surface receptor, the major protease of CoV3Clpro, and the papain-like protease (PLpro) involved in the assembly of new viruses, and RNA-dependent polymerase (RdRp) that facilitates CoV RNA genome replication.[28] The processing and activation of coronavirus S-protein are critical, for the infectivity of the virus. The proprotein convertase family (PCs) is composed of nine serine-secreting proteases and is widely involved in regulating various biological processes in normal and disease states. According to the experts, the biological processing and activation of coronavirus S-protein to expose the reactive domain also explains partially the phenomenon of COVID-19 with severe cardiovascular damage. Key cell entry mechanism includes higher ACE2 (hACE2) binding affinity of the spike, to the receptor-binding domain—reduced dependence on target cell proteases for entry, due to pre-activation by convertase furin.[29] This dependence also makes the virus vulnerable to designer drug interventions.

Researchers from the Department of Statistics, University of Dhaka published a Meta-Analysis, on the prevalence of clinical manifestations and comorbidities of coronavirus infection.[30] Of the total of 33 eligible studies, including 7673 infected patients, the most prevalent clinical symptom was fever (84.49%), cough (56.39%), fatigue (33.65%), dyspnea (22.34%), sputum production (22.34%), and myalgia (16.26%). Other symptoms reported include, shortness of breath, diarrhea, headache, chest pain, vomiting, sore throat, poor appetite, loss of smell and taste, and chills. The most prevalent comorbidity was hypertension (20%), cardiovascular disease (11.9%), and diabetes (9.8%). Other less know comorbidities include, excess weight, obesity, chronic kidney disease, chronic liver disease, chronic pulmonary disease, and cerebrovascular disease.[31-35] These viruses enter the nasal epithelial cells, using the surface spike (S) proteins, to bind a metalloprotease enzyme called, angiotensin enzyme 2 (ACE2), which serve as receptors for 2019-nCoV, on the bronchial epithelial cells and type 11 pneumocytes. Researchers have analyzed the ACE2 RNA expression profile at single-cell resolution. High ACE2 (hACE2) expression has been identified in type 11 alveolar cells of lung, esophagus, enterocytes of ileum and colon, cholangiocytes, myocardial cells, kidney proximal tubule cells, bladder urothelial cells, fat cells, and vascular endothelial cells.

Following infection and viral replication, downregulation of ACE2 enzyme occurs, resulting in dysfunction of the angiotensin system, resulting in hypokalemia, vasoconstriction, and development of acute respiratory distress syndrome. The endothelium is the largest organ of the body, covering a large surface area, and reaching out to every tissue and organ. As such, the injury to the endothelium could introduce a cascade of events, leading to platelet activation, thrombin generation, and promotion of both thrombotic and thrombolytic events. Just to distinguish the term 'vascular disease' from the vascular damage and pathology observed in the severely ill Covid-19 patients, we refer to this condition as a 'disease of the blood vessels'. In the majority of cases, the severity of the coronavirus disease has been found to be associated with pre-existing comorbidities, which include metabolic diseases such as hypertension, obesity, diabetes, and vascular diseases. Those with such diseases, or with elevated risk factors for such diseases will have a compromised endothelium, favoring endothelial dysfunction. The infection of endothelium by SARS-CoV-2 seems to add to this problem, by further damaging the endothelium, causing dysfunction, disruption of vascular integrity, and endothelial cell death. These events lead to the exposure of thrombogenic basement membrane and results in the activation of thrombotic and clotting cascade. In view of these observations, critical care clinicians recommend aggressive anti-thrombotic and thrombolytic therapies in the management of acute COVID-19 cases.

There is considerable interest in developing interventions, to prevent the interaction of spike (S) proteins of SARS CoV-2 with ACE2 receptors. According to a Center for Disease Control (CDC) report, the hospitalization rate during the 4-week period (March 2020) was 4.6%.[26] Therefore, major intervention strategies for the treatment and management of coronavirus disease are aimed at less than 5% of the infected population. In this population, the intervention will depend primarily, on clinical manifestations diagnosed by the critical care physicians. Researchers are also working on the interventions, aimed at the prevention of lung injury, protection of endothelium from cytokine storm, and ways and means for promoting effective immune modulation. Phenotype-driven approach to immunomodulation may include anti-cytokine therapy for selective patients and immunostimulatory therapies in others.[36] Researchers from Iran have suggested, the use of mesenchymal stromal cells (MSCs), to combat cytokine storm in covid-patients.[37] They conclude, "In a number of studies, the administration of these cells has been beneficial for COVID-19 patients. Also, MSCs may be able to improve pulmonary fibrosis and lung function."

At the level of population, currently, in the absence of an effective vaccine, the only choice we have is to follow the best

public health practices such as social distancing, using face coverings, or masks, frequent hand washing with soap, contact tracing, and strict quarantine of covid-positive individuals. Taiwan being next door to the epicenter of the global pandemic (China), has done a marvelous job of containing the spread of this virus (443 cases, seven deaths). Singapore has been hailed as a 'winner' of its pandemic response (38, 965 cases and 25 deaths). South Korea's aggressive early response has kept its overall case-count to a minimum (0.02%), of the population (11, 902 cases; 276 deaths), compared to the US 9.24 million cases and 231,000 deaths. In an unprecedented move, the prestigious New England Journal of Medicine has published an editorial with the title, "Dying in a Leadership Vacuum."[38] It starts off with a critical statement, "With no good options to combat a novel pathogen, countries were forced to make bad choices about how to respond. Here in the United States, our leaders have failed that test. They have taken a crisis and turned it into a tragedy." "The responses of our nation's leaders have been consistently inadequate." "Our current leaders have undercut trust in science and in government, causing damage that will certainly outlast them." Furthermore, the US administration has shamefully politicized the premier institutions, such as FDA, CDC, NIH, and the WHO, and has undercut trust in science, and in these prestigious regulatory, and global public health platforms. The editorial concludes, "When it comes to the response to the largest public health crisis of our time, our current political leaders have demonstrated that they are dangerously incompetent. In our half-century experience in academia, we have never seen such a critical editorial, in a prominent journal like the New England Journal of Medicine.

Contrary to these observations, The New York Times (Oct 28, 2020) published an Opinion titled "How America Helped Defeat the Coronavirus (Just not in the United States)." The authors, Sanya Dosani and associates, discuss how the U.S Public health leaders and scientists, have been planning a catastrophe just like COVID-19 for decades, and, in typical American fashion, we didn't just write the pandemic playbook,- we exported it around the world." They conclude, "What we found doesn't change the fact that more than 220,000 Americans have died from COVID-19, but it sheds light on a part of the U.S. pandemic global response. that hasn't gotten a lot of attention: that America's decades of pandemic planning did save lives. Just not at home." Though the 'opinion' of these authors has been presented as a podcast or a video, it basically tells us an investigative story about, - how the American Science and Public Health knowledge was shared worldwide, and gave the wherewithal to various countries around the world, to prepare and fight the viral pandemics. They discuss the success of Vietnam (1173/35), Thailand (3783/59), Republic of Korea (26,271/482), which had the lowest SARS-CoV-2 infection and deaths. They also give credit to the pioneer global health expert Dr. Dennis Carroll, who serves as the Director, Global Virome Project, a global cooperative scientific initiative to massively lower the risk of harm from future viral outbreaks over 10 years. In 2009, after several years at USAID, Dr. Carroll created an agency program called PREDICT, which "tracks what the different viral threats that are in wildlife might look like, what underlying drivers would lead those threats to spill over into the human population."

In the absence of a cure, sensible medicine proposes a gentler, moderate, and humble view of available treatment options and their effectiveness in patients with COVID-19. The approach encourages clinicians, to elevate usual care, reduce unnecessary interventionism, and focus and rely on scientific rigor.[39] By and large, treatment options are based on clinical diagnosis- based treatments for observed symptoms. For patients with COVID-19, who are not hospitalized or who are hospitalized with moderate disease, but do not require supplemental oxygen, -National Institutes of Health (NIH, USA) panel does not recommend any specific antiviral or immunomodulatory therapy, for the treatment of coronavirus disease in these patients. For those hospitalized with severe conditions, the panel recommends Remdesivir 200 mg intravenously (IV) for 1 day followed by a 100 mg dose for four days or until hospital discharge. A combination of Remdesivir and dexamethasone 6 mg IV up to 10 days. As mentioned earlier, there are no US FDA-approved therapies, for the coronavirus disease treatment. Yet when the US president was found to test positive for COVID-19, the prestigious Walter Reed National Medical Center, gave him an infusion of (8.4 grams) an experimental cocktail of Regeneron's REGN-COV2 (REGN-EB3),-two monoclonal antibodies. In addition, (VIP Syndrome), he received both Remdesivir and dexamethasone. He also received zinc, vitamin D, the generic version of Pepcid, and aspirin. Not every patient with a moderate coronavirus disease can afford or will get this kind of treatment.

FDA has created a special emergency program for possible coronavirus therapies, the Coronavirus Treatment Acceleration Program (CTAP). Currently, there are 590 drug development programs in planning stages, 390 trials in review, and five authorized for emergency use—none approved for use in COVID-19 management. There is another challenge, -as mentioned earlier this virus attacks all the tissue, systems, and organs, therefore its infection is accompanied by multiple clinical symptoms. In view of these observations, there is a great opportunity for developing a series of interventions to manage all the associated clinical symptoms. While industry giants are transfixed by the high-stake race to develop a COVID-19 vaccine, an equal crucial competition is heating up, to produce targeted, neutralizing antibodies that could provide, an instant immunity boost against this virus.[40] Immunologist Dennis Burton, whose group at Scripps Research has isolated highly potent monoclonal antibodies against SARS-CoV-2, hopes to move this novel therapy into human studies. He is optimistic that monoclonals will protect people from infection for months with a single shot. Pandemic Prevention Platform (P3) at the Defense Advanced Research Project Agency has a novel approach, in which they aim to develop monoclonal antibodies, that can be made by the body itself, instead of in large fermentation tanks. The idea, which has not been tested in humans for COVID-19, is to inject a person with DNA or messenger RNA, which encodes the desired antibody, allowing their own cells to make it.

According to the most recent WHO report, there are more than 140 COVID-19 vaccine candidates under

development, with a number of these in Phase 3 trials. When a safe and effective vaccine is found, COVAX (led by WHO, GAVI, CEPI) will facilitate the equitable access and distribution of these vaccines, to protect people in all countries. Four COVID-19 vaccine candidates are in Phase 3 clinical testing in the United States, just over eight months after SARS-CoV-2 was identified. This is an unprecedented feat for the scientific community, made by decades of progress in vaccine technology. As of September 28, there are 40 vaccine candidates in human clinical trials. ChAdOx1 no CoV-19, a recombinant adenovirus vaccine developed by the University of Oxford entered human trial in April of 2020 in the UK. Ad5-nCoV, a vaccine developed by CanSino Biologics of Beijing Institute of Biotechnology, entered a human clinical trial in March of 2020. The vaccine "Gam-COVID-Vac," developed by Moscow's Gamaleya Institute, is an adenovirus-based vaccine like the Oxford vaccine. In addition, the second vaccine in Russia, EpiVac Corona, has also been granted regulatory approval. In terms of prevention of SARS-CoV-2, at the population level, there are just two options, other than hiding from the virus by following the best practices recommended by the public health experts. One approach is to hope for the development of herd immunity, and the other is to develop a safe and effective vaccine and vaccinate most of the population. According to the experts, both these approaches require at least 60% of the population to acquire reasonable immunity to the virus. Researchers from the School of Public Health, Imperial College of London, have reported that antibodies developed in response to SARS-CoV-2 infection, seem to fade away in significant proportions, in just 3 or 4 months. This is not at all the good news. Having said that, we need to remind the reader that doesn't mean that immunity, either induced by infection or by vaccination, is necessarily short-lived. Memory cells can indeed respond to and combat a new infection, as programmed by innate physiological responses.

Currently, there are at least 51 studies listed in the COVID-19 vaccine tracker of the Regulatory Affairs Professional Society (RAPS) site. The top ten entries which are under Phase 3 trial include Ad5-nCoV, a recombinant vaccine by CanSino Biologics (China); AZD1222, a replication-deficient adenovirus vector vaccine (The University of Oxford, the Jenner Institute); CoronaVac by Sinovac; JNJ-78436735, a non-replicating viral vector by Johnson and Johnson; mRNA-1273, an mRNA based vaccine by Moderna; an unnamed inactivated vaccine by Wuhan Institute of Biological Products; NVX-CoV2373, a nanoparticle vaccine by Novavax. There are several new entries in Phase 2/3 trials including, BCG vaccine by the University of Melbourne and Mass. General Hospital, Boston; BNT162 mRNA-based vaccine by Pfizer, BioNtech; and Covaxin, an inactivated vaccine by Bharat Biotech, National Institute of Virology, India. According to a recent article in the New Engl J Med,[41] confidence in any COVID-19 vaccine that is made available under an emergency authorization (EUA), will depend on the rigor of the clinical criteria, including the duration of follow-up, safety, and efficacy of the vaccine. With Phase 3 clinical trials of COVID-19 vaccine underway, safety and efficacy data will be provided, to the FDA soon after they are compiled.[42] Emergency Use Authorization will be made by the FDA's Center for Biologics Evaluation and Research (CBER). The decision of this branch of the FDA has been approved by the Vaccines and Related Biological Products Advisory Committee (VRBPAC). In a short overview like this, on a complex topic like the COVID-19 syndemic, it is difficult to cover all aspects of the disease. We urge readers to refer to original articles, reviews and professional guidelines and COVID-19 resources.[22,43-53]

Conclusion

SARS-CoV-2-2019—the most pathogenic killer virus—has created an unprecedented, once in a century syndemic. Currently, global COVID-19 positive cases exceed 43,623,111 with 1,161,311 deaths. In the most advanced nation, the US, the COVID-19 positive cases exceed 9.2 million with 230,000 deaths. According to the experts, the coronavirus pandemic is the greatest threat, to prosperity and well-being, the US has encountered since the Great Depression. This is true for the other nations as well. This observation has led Harvard Economists, to estimate the collective cost of this pandemic, and publish their viewpoint in JAMA—"The COVID-19 Pandemic and the $16 Trillion Virus."[10] Coronaviruses are relatively large viruses (125 nanometers) with over 29, 000 genetic bases. They are bestowed with a genomic proof-reading mechanism, which keeps the virus from accumulating unwanted mutations. Analysis of 48,635 samples, confirms a low mutation rate of the virus, with an average of 7.23 mutations per sample. Further studies combining genomic details, with epidemiological information and clinical features of COVID-19 patients, may be extremely useful to identify strategies and therapies that can help to reduce the burden of this disease. For instance, one study has shown that the D614G mutation in the Spike protein may be associated with higher case fatality rates. These viruses continue to evolve, surely new features will emerge, or mutate alongside the genomic sequences, with clinical and pharmacological repercussions. Constant monitoring of mutations, therefore, will also be pivotal in tracking the movement of virus, between individuals and across geographical areas.

The virus latches onto a receptor called ACE2, which is found on the lining of the arteries and veins, which are the major supply routes, to all the organs of the body. Yet another enzyme, 'Furin" also seems to play a role in cleaving the viral spike protein. Both enzymes ACE2 and furin are abundant throughout the body, and facilitate the transmission of the virus from cell to cell, as well as person to person. Furin is known to be involved in the cleavage of a wide variety of proteins and is expressed ubiquitously. Recent findings suggest yet another receptor, one called neuropilin-1 facilitates virus entry into cells. Once the spike protein is attached, the internalization of the virus is promoted by hemagglutinin cleavage, modulated by the TMPRSS2, a cell surface-expressed protein by epithelial cells. Once the virions thus released fuse with the membrane, ACE 2 expression seems to get downgraded, resulting in excess production of angiotensin, and enhancing

oxidative stress mechanisms. Of the total of 33 eligible studies, including 7673 infected patients, the most prevalent clinical symptom was fever (84.49%), cough (56.39%), fatigue (33.65%), dyspnea (22.34%), sputum production (22.34%), and myalgia (16.26%). Other symptoms reported include, shortness of breath, diarrhea, headache, chest pain, vomiting, sore throat, poor appetite, loss of smell and taste, and chills. The most prevalent comorbidity was hypertension (20%), cardiovascular disease (11.9%), and diabetes (9.8%). Other less know comorbidities include, excess weight, obesity, chronic kidney disease, chronic liver disease, chronic pulmonary disease, and cerebrovascular diseases.

By and large, the treatment options for the effective management of COVID-19 positive individuals are clinical diagnosis-based and dependent on observed clinical symptoms. For patients with COVID-19, who are not hospitalized or who are hospitalized with moderate disease, but do not require supplemental oxygen, the National Institutes of Health (NIH, USA) panel does not recommend any specific antiviral or immunomodulatory therapy, for the treatment of coronavirus disease in these patients. In the eight months that have passed since the outbreak of this novel virus, emergency medicine staff, critical care clinicians, and other health care experts have learned a lot about the transmission, transfection, pathogenicity, and the role of preexisting health conditions, in enhancing the severity of this disease. This collective knowledge has helped the health care workers, to provide better care for the COVID-19 positive individuals. In the US, 4 vaccine candidates are in Phase 3 clinical trial, with initial results expected soon. There is considerable hesitation about receiving COVID-19 vaccination in the general public. Reasons include, the novelty and rapid development, as well as politicization of the pandemic, and inconsistent messages from scientists and government leaders. It is critical that clinicians stay well informed about emerging data, safety and efficacy of drugs, as well as these vaccines so that they can help patients make sound decisions.

Finally, we would like to encourage readers to watch the video released by the authors of the New York Times 'opinion' titled, "How America Helped Defeat the Coronavirus (Just not in the United States)" and the editorial published by the New England Journal of Medicine titled "Dying in a Leadership Vacuum."

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COVID-19 Infection

BRIEF REVIEW

INTERNATIONAL JOURNAL OF BIOMEDICINE

Obstructive Sleep Apnea and COVID-19 Infection Comorbidity: Analysis of the Problem in the Age Aspect

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Abstract

In the literature, there are suggestions of a possible mutual influence between obstructive sleep apnea (OSA) and the novel coronavirus disease 2019 (COVID-19).The aim of this review is to analyze data found in the literature related to pathogenetic aspects of the impact of OSA on COVID-19, and vice versa, and features of the course of this comorbidity in both adulthood and childhood. Information was searched in MEDLINE, PubMed, EBSCO, and RSCI databases, which presented some data for the connection between COVID-19 and OSA, as well as aspects of OSA and COVID-19 comorbidity for adults and the elderly. The common pathophysiological pathways leading to increased systemic inflammation and adverse consequences of OSA and COVID-19 infection do coexist and were revealed in detail. We paid special attention to aspects of this comorbidity in children, and found that research in this area was lacking. Based on this information, it should be concluded that: 1) more pediatric studies of links between OSA and COVID-19 are urgently needed; 2) screening hospitalized COVID-19 patients with tools to detect OSA could identify those at risk for a severe course of these diseases and adverse outcomes; 3) treating OSA will allow increasing the likelihood of developing a robust and long-lasting post-COVID-19 adaptive immunity in these patients. (**International Journal of Biomedicine. 2020;10(4):312-315.**)

Key Words: obstructive sleep apnea • COVID-19 • comorbidity • pathogenesis • adults • children

Just over half a year has passed since the World Health Organization (WHO) declared the COVID-19 outbreak a public health emergency of international concern. At the time, there were only 100 cases outside of China and no deaths. Since then, the incidence has been increasing exponentially, affecting every country in the world. WHO data, published on August 16, 2020, reported 21,294,845 cases of COVID-19 worldwide, with 761,779 deaths, but on September 21, 2020, reported 30,949,804 cases in 235 countries, and 959,116 deaths.[1] The number of new cases of COVID-19 increases week by week and reaches 2,000,000 per week (Fig.1).[2] In the Russian Federation as of September 21, 2020, the number of cases of COVID-19 infection was 1,109,595, with 6,196 deaths.[3] The COVID-19 pandemic has placed an enormous burden on the global health care system with numerous consequences. The secondary effects of this pandemic, along with major disruptions to essential health care services, are having a huge impact on politics, the economy, and people's daily lives.

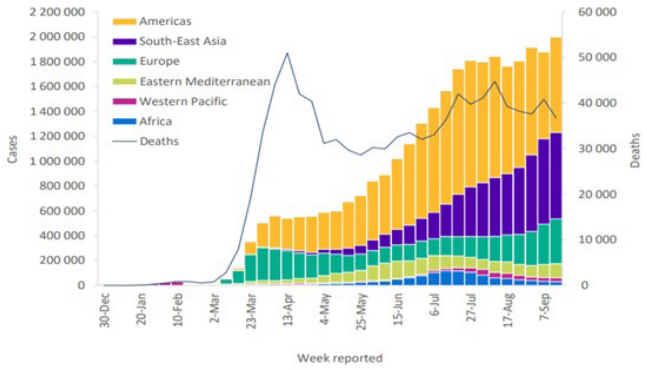

Fig. 1. *Number of COVID-19 cases reported weekly by worldwide and global deaths, December 2019 through September 2020 (adapted from (2).*

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COVID-19 was predominantly more prevalent among adults and the elderly at the beginning of the outbreak, and the percentage of confirmed cases among children and adolescents was relatively small. The Chinese Novel Coronavirus Pneumonia Emergency Response Epidemiology Team reported that about 2% of 44,672 confirmed cases of COVID-19 in China through February 11, 2020, were children between 0 and 19 years of age;[4] of these, 0.9% were subjects younger than 10 years old. One month later in Italy, Livingston et al. found that 1.2% of all confirmed cases (22,512) of COVID-19 were children, with no deaths.[5] At the same time, of 4,226 COVID-19 cases detected in the United States, 5% were children.[6] Importantly, more than 90% of ill children were asymptomatic or had mild or moderate symptoms of COVID-19 infection. Since then, the number of child COVID-19 cases has increased significantly, especially in younger age groups, and the disease increasingly began to manifest with so-called «pediatric multisystem inflammatory syndrome»[7] with severe outcomes;[8] therefore, high attention and care should be directed to children and adolescents with COVID-19, just like adult patients. It was shown that COVID-19 is an acute, self-limiting disease, but some patients can die, according to Xu's et al. reported mortality rate of 2% in adults.[9] The Centers for Disease Control and Prevention (CDC) has published data of deaths involving COVID-19 with a focus on ages 0-18 years in the United States, and on August 26, 2020, reported that in the US there were 29 deaths among children aged 0-4 and 65 among those aged 5-18 years.[10]

Some studies have reported a higher COVID-19 incidence in men than in women (0.31/100,000 vs 0.27/100,000, respectively).[11] Wherein, the case mortality rate for males was also higher than for females (2.8% vs 1.7%, respectively), with a similar trend in children and adolescents, reported by the CDC in the US.[10] It has also been proven that the risk of infection and complications increases significantly in the presence of comorbidities (both adults and children): Patients who reported no comorbid diseases had lower mortality rates than those with comorbid conditions, such as cardiovascular diseases, diabetes, chronic obstructive pulmonary disease, or cancer.[4,12]

All of the above-mentioned prompted us to closely study the features of the pathogenetic mechanisms and relationships between COVID-19 and one comorbidity, namely OSA, that is very important for the health care of both adults and children. It should be noted that, despite the obvious relevance and severity of this problem worldwide and numerous discussions in the foreign literature, there are many works of Russian scientists about COVID-19 infection and coexisting diseases in adults,[13,14] while only one publication is devoted to the association between COVID-19 and OSA.[15] But there are no Russian publications, and just a single foreign one, on this problem in pediatric patients.

The aim of this review is to analyze data found in the literature related to pathogenetic aspects of the impact of OSA on COVID-19, and vice versa, and features of the course of this comorbidity in both adulthood and childhood.

Information was searched in MEDLINE, PubMed, EBSCO, and RSCI databases.

OSA and COVID-19 infection in adults and the elderly

By definition, OSA is a condition characterized by recurrent episodes of upper airway obstruction during sleep, due to anatomical narrowing of the airway, arousals, and recurrent nocturnal intermittent hypoxemia.[16] OSA is common and the incidence is increasing worldwide. Interestingly, the rate of the condition has a gender dimorphism, as in COVID-19. It is estimated that OSA affects 27% of men and 11% of women in the middle-aged adult population.[17] This disease is significantly underdiagnosed, due to lack of awareness and its insidious course, and often remains untreated.

OSA is strongly associated with COVID-19 comorbidities, namely cardiovascular disease, hypertension, diabetes, and obesity.[18] Studies published from April to September 2020, worldwide, on adult and elderly subjects highlighted the strong overlap between OSA and the risk factors for adverse outcomes of COVID-19 infection.[19-27] It is known that, both in patients with OSA and COVID-19 infection, there are associations with increased systemic concentrations of IL-6, IL-17, TNF-α and other pro-inflammatory mediators (e.g., "cytokine storm")[28,29] as well as oxidative stress (inevitably accompanying OSA and coexisting diseases),[30,31] which confirms the possibility of potentiating systemic inflammation in the case of their comorbidity and acts as a major determinant of the adverse consequences of these coexisting diseases (Fig.2). Suen et al. has reported that OSA can potentially aggravate inflammation in COVID-19–related sepsis or acute respiratory distress syndrome.[32] The CORONADO study showed highly significant associations between OSA and the risk of death on day 7 of COVID-19 disease (adjusted OR 2.65).[33] Cade et al. also described OSA as a risk factor for COVID-19 mortality and severe morbidity, highlighting the need for close monitoring of patients with OSA who become infected.[34]

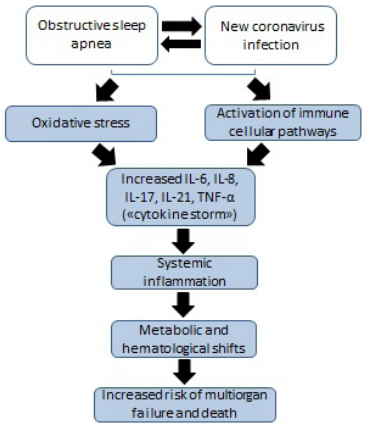

Fig. 2. *The common schema illustrating of a cascade of pathophysiological pathways that result in increased systemic inflammation and adverse consequences if obstructive sleep apnea and COVID-19 infection are comorbidity.*

There are suggestions of possible links of mutual influence of OSA and COVID-19. OSA could be also added to the severity of the pulmonary inflammatory process of COVID-19 infection. An increased incidence of OSA in COVID-19 patients through the dysregulation of the renin-angiotensin system and hypertension,[35] as well as hyperinflammatory response,[32] which can occur in COVID-19 pneumonia, can

cause multiorgan failure.[36] Furthermore, management of OSA patients with COVID-19 infection requires special care and precautions.[37,38]

Children's aspects of OSA and COVID-19 comorbidity

It is well known that OSA affects 1%-5% of all children and adolescents [39] and is associated with COVID-19 comorbidities (e.g. hypertension and obesity), as in adults and the elderly,[40-42] which along with the appearance of COVID-19-induced lung injury in children and an almost equal OSA and COVID-19 incidence in the pediatric population, is a serious problem for children's healthcare services. Despite increasing evidence showing children have more mild presentations and better outcomes with COVID-19, there is very limited documentation in the medical literature of apnea cases, especially coexisting OSA, potentially aggravating the course of the disease. It has been reported that sleep-disordered breathing in children and adolescents with COVID-19 may manifest by episodes of apnea. In three studies, Needleman et al.,[43] Brabin et al.,[44] and Enner et al.[45] described COVID-19-associated apnea and circumorally cyanosis in a 3-week-old, full-term male infant; and neonatal apnea as initial manifestation of COVID-19 infection (a type of apnea was not marked: central or obstructive in both cases), and COVID-19-associated encephalopathy characterized by focal seizures and central apnea in a 14-year-old girl. We found information about a 3-year-old boy with Down syndrome and many comorbidities, including OSA, with dependence on continuous positive airway pressure (CPAP), who was infected by COVID-19, prompting development of mild hypotension and the need for an increase in CPAP.[46] Today, this is perhaps the only finding about a case of COVID-19 infection in a pediatric patient with OSA as a chronic lung disease.

Conclusion and outlook

Based on our analysis, it can be concluded that such a serious problem as COVID-19 infection against the background of coexisting diseases, namely chronic respiratory pathology, for example OSA, in adult and elderly patients is being carefully studied and widely discussed by scientists from many countries. However, there are few studies in the pediatric population are single, which urgently necessitates research into links between OSA and COVID-19 in childhood and adolescence. It should also be remembered that screening hospitalized COVID-19 patients with tools to detect OSA (subjective or/and objective, such as polysomnography) could identify those at risk for adverse outcomes, and by subsequently restoring adequate sleep, we can not only reduce the incidence of complications in such patients but also increase the likelihood of developing a robust and long-lasting post-COVID-19 adaptive immunity.

Competing Interests

The authors declare that they have no competing interests.

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REVIEW ARTICLE

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Functional Bowel Disorders and Obesity in Children: State of the Problem

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Abstract

Obesity and functional bowel disorders (FBDs) are often observed in children and have common risk factors. The present review aimed to summarize the published data on the association between obesity and FBDs in children and a discussion of possible pathophysiological mechanisms that may be involved. Published data indicates that obesity and FBDs could be associated conditions. There is substantial evidence that obesity in children is associated with constipation. However, it should be noted that there were few studies in this direction, and those studies were heterogeneous in both the composition of participants and studied diagnostic criteria, and in the majority of cases, they were not adjusted for potential confounders. The association between obesity and FBDs can be explored through diet, peculiarities of eating behavior, and psychological factors. The most promising direction in the study could be the study of the gut microbiota, the changes in which can contribute to the development of immune dysfunctions of the bowel, chronic low-grade inflammation, increased colonic permeability, motility disturbances, and visceral hypersensitivity. The studies in this area can provide important data for developing a strategy of treatment and prevention of both groups of diseases. (**International Journal of Biomedicine. 2020;10(4):316-323.**)

Key Words: irritable bowel syndrome • functional constipation • adolescents

Abbreviations

BW, body weight; **GM**, gut microbiota; **FBD**, functional bowel disorders; **FGIDs**, functional gastrointestinal disorders; **IBS**, irritable bowel syndrome; **FC**, functional constipation.

Obesity in children is one of the most serious problems in public healthcare of the 21st century. From 1980 to 2013, the number of adults who were overweight or obese increased by 27.5% and children – by 47.1%.[1] The results of a multicenter study in Russia showed that around 20% of children were overweight and 5% suffered from obesity.[2] It is a concerning fact that children with obesity continue to suffer from it in adult life. If the existing tendencies remain, around 20% of infants with obesity will turn into children with obesity, 40% of children with obesity will turn into adolescents with obesity, and 80% of these adolescents will inevitably become adults with obesity.[3] Comorbid conditions with obesity include a wide spectrum of chronic diseases that involve nearly all organs and can exert a significant long-term negative effect on health and life expectancy. Thus, in 2015, there were 4 million obesity-related deaths and 120 million disability-adjusted life years in the world.[3]

Even though the main efforts of researchers are focused on the study of the associations between obesity and such potentially life-threatening conditions as diabetes mellitus, cardiovascular diseases, and nonalcoholic fatty liver disease, recently, data has appeared that shows an association between obesity and functional gastrointestinal disorders (FGIDs) in adult and pediatric populations. FGIDs are a heterogeneous group of recurrent abdominal symptoms, the origin of which does not have a structural or biochemical explanation.[4] They are widespread in the pediatric practice, i.e. 50% of visits to pediatric gastroenterologists and 2-4% of general pediatric visits.[5]

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Rome IV criteria approved in 2016 divided pediatric FGIDs into two groups: group G that included disorders observed in children from birth to 4 years old (not included in the review) and group H that included disorders in children older than 4 years and adolescents. The last group contains three categories: functional disorders associated with nausea and vomiting, functional disorders associated with abdominal pain, and functional defecatory disorders.[6] FBDs that are characterized by a change in the rate of defecation and stool consistency, as well as IBS and FC, are the most common reasons for seeking treatment.

The majority of studies dedicated to the association of obesity and FBDs were conducted in adult cohorts. Pediatric studies in this area are sparse and provide controversial results. Along with that, obesity and FBDs seem to have more in common than a high incidence rate in the population. In the pathogenesis of both conditions, there are factors of diet and lifestyle, psychological impairments, and altered GM. Thus, an understanding of the true associations between obesity and FBDs in pediatric patients is important for providing key information for the development of pathogenetically grounded strategies of treatment, prevention, and early diagnostics for both groups of diseases.

Thus, the present review aimed to summarize the published data on the association between obesity and FBDs in children and a discussion of possible pathophysiological mechanisms that may be involved.

Definitions

It should be noted that the definition of FBDs in children and adolescents changed after the approval of the Rome IV criteria.

IBS is diagnosed in children with abdominal pains that persist for not less than 4 days per month. At the same time, they are associated with defecation, change in the rate and consistency of stool, and in combination, these symptoms cannot be explained by other medical conditions. The specified complaints should persist for not less than two preceding months. Based on the prevailing stool shape, IBS is divided phenotypically into IBS with constipation (IBS-C), IBS with diarrhea (IBS-D), a mixed subtype of IBS and IBS without a subtype.[7] However, it should be noted that in 24% of children, the subtype of IBS can change within 12 months.[8] This could be associated with the fact that the majority of studies on IBS in pediatrics do not consider separate subtypes of this disease and look into IBS as one group.

FC in children older than 4 years old is diagnosed in cases with two or more symptoms, provided they are observed not less than one time per week for minimum of one month: two or fewer defecations per week; at least one episode of fecal inconsistency per week; a history of retentive posturing or excessive volitional stool retention; a history of painful or hard bowel movements; the presence of a large fecal mass in the rectum; a history of large-diameter stools that may obstruct the toilet. At the same time, the specified symptoms cannot be explained by other medical causes.[9]

It should be highlighted that according to the Rome IV criteria, abdominal pain does not exclude FC. However, in children with FC, unlike in children with IBS, constipation can be the main symptom. Pains disappear after FC resolution and are considered to be secondary symptoms.[9] Thus, the separation of FC and IBS-C requires thorough detailing of complaints, which is not always possible in pediatric patients. The described facts and constant evolution of diagnostic criteria of FGIDs, which has been ongoing since 1990, significantly hamper their unification for systematic reviews and meta-analyses.

Obesity and FBDs in children: Is there an association between them?

Studies that have focused on the association between FBDs and obesity can be divided into two categories: studies among patients of specialized clinics and centers, and studies performed among samplings of the general population.

The association between FBDs and obesity in children and adolescents has been primarily studied among gastrointestinal patients. Two studies performed at gastrointestinal centers in the USA (n=375) and Israel (n=274), with 10 years between them, that included population control groups showed that there was a significant association between these diseases despite different variants of Rome criteria used for to verify the diagnosis.[10,11] The study performed by Pawłowska et al.[12] showed that IBS in pediatric patients was associated not only with excessive BW but also with the share of fat mass in the body that was assessed using bioimpedancemetry.

The rate of IBS among children with obesity was studied once, and the results showed the rates to be significantly higher than in the control group (10.67% vs 2.60%; P=0.024).[13]

The authors managed to find only a single, but well-designed, study that focused on the incidence rate of FGIDs, including IBS and FC, in the sampling of healthy children aged 4-18 years old (n=450). The study was conducted in a general pediatric clinic without a bias towards either obesity or FGIDs, which would inevitably occur at a specialized center. That study was unique because it accounted for such possible confounders as the age of the participants, their ethnicity, and the area where the participants were included in the study. The obtained results confirmed that children with obesity were more prone to the development of FGIDs, and in particular IBS, than children with healthy BW.[14]

An association between obesity and constipation has been studied more actively but the obtained results are more controversial.

In 2004, Fishman et al.[15] were the first to report that obesity and FC are associated conditions. In further years, three studies performed in the USA showed that there was a higher incidence rate of obesity among children with constipation than among healthy children in the control group of the general population.[10,16,17] Two of these studies showed that the association between obesity and constipation was more significant among boys.[16,17]

The association between weight status and FC was reported in three studies, which were conducted among children with obesity.[13,18,19] Two studies, which used Rome III criteria to verify the diagnosis, presented comparable data on the rate of constipation in children with obesity (18.44% and 21%, respectively).[13,18] In the third study, the authors did not use diagnostic validated criteria, and the rate of constipation in

adolescents with obesity was 38.4%.(19%) It should be noted that only one of the above-mentioned studies had a control group (23% vs 13%).[14]

We revealed only one study conducted among the general population, which found that children with overweight or obesity suffered from constipation more frequently than children with healthy BW.

On the other hand, several studies did not confirm the association between obesity and constipation. Among the studies that were performed in clinics, there was one conducted by Çağan Appak et al.[20] that showed that BMI z-scores of children with FC were comparable with the control group. Another "case-control" study showed that obesity and being overweight were more common in children with FC, than in children that had other diseases(18% vs 12% and 33% vs 23%, respectively), but these differences were not statistically significant.[21]

Three populational studies, two of which were conducted among adolescents in Brazil and one in Columbia (2820 participants aged 8-18 years old),[22-24] also did not reveal an association between obesity and FC, though, in one of them, girls with constipation tended to have a higher BMI than girls that did not suffer from constipation.[24] Kiefte-de Jong et al.[25] published the results of the observation for the population cohort by year of birth (n=2420). They did not reveal a significant association between excessive weight and constipation in 4-year-old children.[25]

Finally, a high-profile study that included 14,626 Taiwanese adolescents showed that there was a reverse association between rare defecations (<3 times per week) and being overweight or obese.[26] Although rare defecations do not completely characterize constipation, the association between FC and a deficit of fat mass presented by Pawłowska corresponded to these results.[12] The available data indicate that weight loss may modestly improve both upper and lower abdominal GI symptoms, thus suggesting a potential causal link.[27]

Few studies characterize the peculiarities of clinical manifestations of FBDs in children with obesity. Teitelbaum et al.[10] showed that among all the subtypes of IBS, only IBS-D was associated with obesity in the sampling of gastroenterological patients aged 2-20 years old. This agrees with the results of the study by Gurova et al.,[28] which showed that patients aged 12-15 years old with obesity more often had unformed stool and a higher rate of defecations (up to 12-13 times per week) than adolescents who were merely overweight.

Bonilla et al.[29] monitored the dynamics of 351 children with FGIDs associated with pain (including IBS) and revealed that obesity significantly increased the possibility of persisting abdominal pains. In children with obesity, abdominal pains occurred more often and were more intense, and they led to absence from school and routine life disturbances. When it comes to constipation, Misra et al.[17] showed that the rate of defecations in general and the rate of painful defecations in children with FC did not depend on body weight. The same study showed that constipation in patients with obesity was more difficult to treat. There is evidence that FC in children with obesity was associated with episodes of fecal incontinence.[15,22] However, other studies did not confirm this association.[16,17]

To sum up, the published data present quite uniform conclusions on the association between obesity and IBS but controversial conclusions on the association between obesity and FC. However, even though the association between obesity and IBS was confirmed by all the conducted studies, their conclusions have some limitations. First, these studies did not include large samplings of the population and most of them did not have sufficient power. Second, they used different diagnostic criteria for FGIDs. Third, they did not consider possible confounders, like diet and peculiarities of eating behavior, that could be more significant for IBS than obesity itself. Regarding childhood obesity and FC, the association between these diseases was confirmed in the majority of studies conducted in specialized clinics, but several studies in the general population provided neutral and even directly contrasting results. This may be because of a selection bias due to the fact that patients with more severe clinical manifestations of both FBDs and obesity seek medical care, and the associations between FBDs and obesity in clinical groups may be different than in the general population. Moreover, in some studies, there were no control groups and the rate of FC was compared with some average populational values. Along with that, the rate of FC could be significantly different not only between different regions but also between different regions of one country.[23] A systematic review by Koppen et al.[30] in 2016 that focused on the studies between functional defecation disorders and excessive weight in children did not provide consistent results because of heterogeneous designs, low quality of the reviewed studies, and controversial results that were obtained by the authors.

All the above-mentioned facts indicate that despite its significance, the problem of the association between obesity and FBDs remains unresolved.

Obesity and FBDs in children: Possible mechanisms of association

Some associated factors could be considered as causes of obesity in children and adolescents. For one, the qualitative and quantitative content of a diet plays an important role in the development of obesity and symptoms of FBDs. Therefore, the peculiarities of a diet that contribute to the development of both conditions can be treated as connecting links between them.[31] Potentially, a tendency towards the consumption of products with a low content of fiber (which stimulates the osmolarity of stool), high content of saturated fats (which stimulate the motility), and high content of fermentable carbohydrates can contribute to the development of symptoms of FBDs in these patients.[32,33] In a major cohort in France, Schnabel et al.[34] studied the association between the consumption of ultra-processed food that was characterized by a high content of fatty acids, sugar, and sodium, and a low content of fiber and FGIDs. The results showed that an increase in the consumption of ultra-processed food was associated with a significant risk of developing IBS and was also associated with a higher IBM of the participants.

The role of excessive consumption of fermentable

carbohydrates, such as fructose, lactose, fructans, galactans, etc., in the development of the symptoms of FBDs has been actively studied in recent years. These short-chain carbohydrates contained in a wide range of food are poorly absorbed in the intestine, and their fermentation by the GM leads to the formation of carbon dioxide, hydrogen, and/or methane.[35] Small osmotically active molecules that are formed during the fermentation attract liquid to the small intestine and can contribute to the development of osmotic diarrhea, symptoms of bloating, pain, and discomfort.

Excessive consumption of poorly absorbable sugars, in particular, corn syrup with fructose, which are widely consumed in the Western diet, can partially explain the association between obesity and FBDs shown in some studies.

Ozaki et al.[36] revealed malabsorption of fructose in 67.7% of children aged 4-14 years old with functional abdominal pain, including in 90% of children with IBS included in the study. Children with malabsorption of fructose consumed more carbohydrates and high-**energy foods** and had higher values of BMI Z-scores. There are few studies dedicated to the selective impact of fermentable carbohydrates in forming the association between diet and the symptoms of FBDs in patients with obesity. However, the results of double-blind, randomized, controlled studies showed the effectiveness of a diet with a low content of fermentable carbohydrates in reducing gastrointestinal symptoms in a majority of children with IBS. This can indirectly indicate the role of these carbohydrates in the development of FBDs.[37,38] Still, because of the limited scope of such a diet, studies on its long-term influence on the growth and development of children are required before it could be widely recommended in clinical practice.

An excessive amount of fats in the diet can be also associated with symptoms of FBDs in patients with obesity.[39] Out of all nutrient ingredients, lipids are the strongest modulators of the gastrocolic reflex.[40] It is known that lipids in food weaken the motility of the intestine, slowing down the transit of gas via the intestine and leading to abdominal bloating; on the other hand, they enhance the motility of the colon, contributing to the development of diarrhea.[39] In a study by Levy et al.,[41] the content of fats in the diet of adult patients with overweight and obesity had an independent positive association with an increase in the rate of stool and diarrhea in the multivariate model.

The content of fiber in a diet is associated with constipation, and the studies we reviewed provided controversial results. Çağan Appak et al.[20] did not reveal a significant difference in the daily consumption of fiber between healthy children and patients with FC. At the same time, Macêdo et al.[24] demonstrated that the low content of fiber in a diet was associated with constipation in female adolescents (OR=3.42; 95% CI: 1.08-12.06). Also, BMI in girls with constipation was higher than in girls without constipation (P=0.001), which does not exclude the fact that consumption of fiber is one of the modifiers of the association between BMI and FC.

Systematic reviews of pediatric studies did not provide evidence that would allow the specialists to recommend the consumption of fiber that exceeds the daily norm for the weight and age of a child for the treatment of FC.[42] Nevertheless, the above-mentioned data could be a useful recommendation for patients with obesity and FC to consume a sufficient amount of dietary fiber,[43] which can be important in the prevention and treatment of both diseases.

One of the possible explanations of the association between FBDs and obesity can be pathological patterns of eating. It has been shown that impairments of eating behavior, such as compulsive and episodic overeating and different variants of limiting behavior, were common problems among children with obesity.[44] The results of the meta-analysis of 36 studies showed that more than a quarter of children and adolescents with overweight and obesity suffered from compulsive uncontrolled overeating,[45] which can contribute to the development of gastrointestinal symptoms. A great volume of food leads to quick gastric distention and further osmotic bolus to the intestine, which increases the capacity of physiologic adaptation.[46] A study by Levy et al.[41] showed that compulsive overeating had a significant association with abdominal pain and bloating in the multivariate models.

Obesity was associated not only with behavioral eating disorders but also with such psycho-emotional impairments as anxiety and depression.[47,48] A similar spectrum of psycho-emotional impairments observed in children with FBDs[49-51] can indicate that obesity and FGIDs had similar psychophysiological mechanisms. A longitudinal survey by Koloski et al.[52] showed that elevated levels of anxiety in participants that did not initially have FGIDs were significant predictors of the development of FGIDs within 12 years of the observation. In this study, the predictors of the development of IBS were elevated levels of anxiety and depression. The mechanisms that link obesity, FBDs, and psycho-emotional impairments require further studies, but it is suggested that the hypothalamic-pituitary-adrenal axis is one of the key links in the pathogenesis of obesity and that this axis also takes part in the pathogenesis of FBDs.[53]

Iovino et al.[54] used a rectal barostat to reveal that emotional instability modulated visceral perception and could contribute to the formation of visceral hypersensitivity and abdominal pain in children with obesity. It was also shown that among patients with morbid obesity, those who suffered from IBS had significantly higher levels of fatigue, anxiety, and depression as well as a lower quality of life.[55]

One of the connective links between obesity and disturbances in the rate and character of stool could be peculiarities of gut transit. A shorter time of colonic transit in relatively healthy people with obesity can be one of the explanations of the association between obesity and diarrhea in the study that included the adult population.[56]

Bouchoucha et al.[57] conducted a study that included 354 patients with constipation. The study not only obtained similar results but also showed a reverse association between the time of rectosigmoid transit and age. The authors suggested that children should have the maximal expression of the transit delay in the rectosigmoid region. Physiological age-related alterations in colonic transit can be associated with the neuropeptide profile of the gut, change in the content and volume of interstitial cells of Cajal,[58] and peculiarities of the microbiological landscape. The fact that the mechanisms that form the association between FBDs and obesity that involve

gut transit differ in different periods of life, could be one of the explanations for the controversial results obtained during the studies on the association between obesity and FC that included children and adolescents. The age-related range (usually 4-18 years old) can lead to a shift in the distribution and affect the study results. This suggestion is proven by the fact that the results of the studies that focused on adolescents were comparable with the ones that involved adults.[29] The study of cohorts by the year of birth that included 26-year-old New Zealanders (n=980) revealed a significant inverse association between BMI and diarrhea, which was comparable to the results of the majority of studies that included adults.[59]

In addition to the above-described facts, obesity and FBDs have another potential common pathophysiological factor that attracts researchers – GM. On the one hand, changes in the content of GM are associated with the onset and persistence of FBDs. On the other hand, they were described as an important determinant of energetic metabolism and obesity.[60]

The GM are responsible for the fermentation of undigested proteins and carbohydrates, utilization of hydrogen, and transformation of bile acids, gases, and secondary bile acids. Metabolic functions of the GM can contribute to disturbances of the intestine's immune function, chronic low-grade inflammation, increased colonic permeability, motility disturbances, and visceral hypersensitivity.[7]

Some studies showed that patients with obesity had an increase in the ratio of Firmicutes/Bacteroidetes, which was associated with an enhanced capacity to derive energy from food due to a more active breakdown of carbohydrate complexes.[61,62] Even though the data on the content of microbiota in patients with IBS is controversial, these patients have a similar type of dysbiosis.[63] Saulnier et al.[64] showed that the content of microbiota correlated with the severity and rate of abdominal pain in children with IBS. Indirectly, the role of GM in the pathogenesis of FBDs can indicate the effectiveness of treating these diseases with probiotics. A meta-analysis of three randomized placebo-controlled studies revealed a significantly higher rate of response to the treatment (resolution of weakening of pains) in children with IBS that received *Lactobacillus rhamnosus* GG containing probiotics, in comparison with a placebo.[65] Clinical improvement was also observed in the treatment of children with IBS with probiotics that contained VSL#3 and a combination of three bifidobacteria.[66,67] The mechanisms of the positive influence of probiotics on the symptoms of IBS are to be investigated. It is also unclear how the presence of obesity affects the clinical profile of IBS in probiotic treatment.

The association between GM and FC is often studied by its influence on gut transit. Even though the pathophysiological mechanisms of this influence are not established, they could be microbial modulation of the expression of genes involved in motor apparatus responses, pH-dependent stimulation of the motility from the products of fermentation, the osmotic effect of metabolites of the microbiota and intestinal distention due to an increase in the production of intraluminal gases (carbon dioxide, hydrogen, and methane) that provoke reflexive smooth muscle contractions.[68] The differences in the GM content between children with FC and the control group were revealed in several studies. The study by Zhu et al.[69] showed that the differences also remained in cases when both groups of comparison consisted of children with obesity. In this study, patients with FC (the average age was 11.8 years old) had a significantly lower level of Bacteroidetes, in particular Prevotella, and the level of some types of Firmicutes, including Lactobacillus, were significantly higher than in children without constipation, while the levels of bifidobacteria were comparable. Other researchers described an increase in the level of bifidobacteria in children with FC.[68,70] The fact that in pediatric practice probiotics usually contain lactobacilli and bifidobacteria, the levels of which are quite high in the microbiome of children with FC, can explain why Wojtyniak's meta-analysis of seven randomized controlled studies that included 515 people did not reveal any benefits from the application of probiotics for the treatment of FC in children.[71]

Conclusion

Published data indicates that obesity and FBDs are associated conditions. The presence of significant associations between obesity and IBS in children was confirmed by all the studies dedicated to this issue. There is significant evidence that obesity in the pediatric cohort is associated with constipation. However, it should be noted that there were few studies in this direction. They were heterogeneous in the composition of participants and applied diagnostic criteria. In rare cases, the data was adjusted for possible confounders and, thus, the association between obesity and FBDs was not obvious.

The above-mentioned facts provide grounds for high-quality studies with unified definitions, diagnostic criteria, precise criteria for study inclusion and exclusion, and a wide range of confounder factors, including diet, psychological, and social-demographic factors. The establishment of a true association between obesity and FBDs would contribute to a better understanding of the complicated biology of both conditions and could optimize approaches to therapy.

The study of GM as a central link that explores the association between eating habits, energy extraction, and weight gain, on one hand, and intestinal motility and visceral sensitivity, on the other, could be quite promising in the study of the association between obesity and FBDs. The studies on GM could help reveal the species of bacteria that contribute to the development of certain types of FBDs, which could provide grounds for the proposed target probiotic treatment.

Competing Interests

The authors declare that they have no competing interests.

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REVIEW ARTICLE

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Some Molecular Mechanisms of Cervical Ripening

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Abstract

Cervical remodeling is an active dynamic process that begins long before the onset of labor. The optimal course of the cervical ripening/remodeling processes is a prerequisite for successful vaginal delivery. Cervical remodeling is a slow progressive process that begins early in mammalian pregnancies, and can be loosely divided into four overlapping phases termed softening, ripening, dilation/labor, and postpartum repair. This review discusses some aspects of structural changes in the cervix at different stages of cervical ripening. In particular, the role of cervical epithelia, immune-inflammatory factors/cells, and components of the cervical extracellular matrix in cervical ripening is considered. A better understanding of the molecular-biochemical and histophysiological processes occurring during cervical remodeling is critical for the development of novel approaches to treat cervical insufficiency, preterm labor, and postpartum cervical disorders associated with its integrity. (**International Journal of Biomedicine. 2020;10(4):324-329.**)

Key Words: cervical ripening • extracellular matrix • histophysiological processes

Abbreviations

AP-1, activator protein 1; **HA**, hyaluronic acid; **GAGs**, glycosaminoglycans; **mRNA**, messenger RNA; **NF-kB**, nuclear factor-κB; **PG**, prostaglandin; **TNFα**, tumor necrosis factor alpha

Introduction

The uterine cervix performs two critical functions during pregnancy. First, the primary biomechanical function of the cervix is to maintain the fetus within the uterus until the appropriate time for delivery. Second, at the end of pregnancy, the cervix prepares for labor and delivery and begins to soften (ripen), thin out, and open (cervical ripening). Studying the ultrastructural processes of cervical remodeling is critical for the prevention and management of preterm delivery.[1-4]

The optimal course of the cervical ripening/remodeling processes is a prerequisite for successful vaginal delivery. The premature cervical opening can result in preterm birth, which occurs in 12.5% of pregnancies and is the leading cause of neonatal morbidity as well as the cause of later health problems.[5] In this regard, understanding the fundamental biochemical and histophysiological processes occurring during cervical ripening is essential in the prevention of preterm labor and birth. This review discusses some aspects of structural changes in the cervix at different stages of cervical ripening. In particular, the role of cervical epithelia, immune-inflammatory factors/cells, and components of the cervical extracellular matrix in cervical ripening is considered.

Distinct Phases of Cervical Remodeling

The transformation of the cervix from a closed rigid structure in a soft and distensible structure, which opens sufficiently for birth, is an active dynamic process that begins at the early stages of gestation. A better understanding

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of the molecular-biochemical and histophysiological processes occurring during cervical remodeling is critical for the development of novel approaches to treat cervical insufficiency, preterm labor, and postpartum cervical disorders associated with its integrity. Although biopsy material from pregnant women before term is limited, experimental studies in rodents have facilitated a comparative evaluation of the cervical remodeling process.

Cervical remodeling is a slow progressive process that begins early in mammalian pregnancies, and can be loosely divided into four overlapping phases termed softening, ripening, dilation/labor, and postpartum repair.[6-9]

Cervical softening (Phase 1)

Cervical softening (Phase 1) can be defined as a change in the biomechanical properties of the cervix when compared with the nonpregnant cervix and is characterized by a progressive decrease in tissue stiffness without loss of tensile strength.[7,10] From a restrictive and rigid barrier before pregnancy, the cervix grows and softens during Phase 1 of remodeling under the trophic influences of a variety of hormones and ovarian steroids.[11] Experimental studies in mice indicated that the softening phase begins by day 12 of a 19-day gestation.[5,7-9,12,13]

Cervical softening begins early in mammalian pregnancies. In 1895, Hegar first described the 'softening' of the lower uterine segment in association with human pregnancy at 4–6 weeks. Phase 1 is a relatively slow and incremental process. This phase takes place in a progesterone rich environment. Yoshida et al.[14] reported that in the early softening period mature cross-linked collagens decline and are replaced by immature collagens to facilitate increased tissue compliance. Increases in collagen solubility in Phase 1 is one of the earlier events in the remodeling process. Akins et al.[15] showed that early changes in tensile strength during cervical softening result in part from changes in the number and type of collagen cross-links and are associated with a decline in expression of two matricellular proteins thrombospondin 2 (THBS2) and tenascin C (TnC).

M. Mahendroo[16] highlights that the gradual replacement of mature cross-linked collagen with collagen harboring reduced cross-links along with the decline in THBS2 and TnC in the cervix is key to modulating collagen architecture within the extracellular matrix during softening and initiating the incremental fall in cervical mechanical strength while maintaining tissue integrity.

Gene expression studies performed by Read et al.[7] revealed a potentially important role of cervical epithelia during softening and ripening in the maintenance of the immuno-mucosal barrier that protects the stromal compartment during matrix remodeling. Expression of two genes involved in repair and protection of the epithelial permeability barrier in the gut (trefoil factor 1, Tff1) and skin (serine protease inhibitor Kazal type 5, Spink5) was increased during softening and/or ripening. Expression of the Pcp4 gene encoding Purkinje cell protein 4 (a neuronal-specific calmodulin regulatory protein that inhibits apoptosis) decreased as remodeling progressed. These results indicate that cervical softening during pregnancy is a unique phase of the tissue remodeling process characterized by increased collagen solubility, maintenance of tissue strength, and upregulation of genes involved in mucosal protection.[7]

A marked proliferation of the mucosal epithelia occurs in the latter half of rat pregnancy.[17] By gestation day 16 and 17 as the softening phase merges into the ripening phase, the epithelium becomes laden with mucin-secreting vacuoles important in immune surveillance and lubrication.[16] During softening and ripening, the cervical epithelia maintain fluid balance and permeability barrier via regulated expression of aquaporins, gap junction proteins connexin 26 and 43, hyaluronan synthase 2, desmogleins (1 alpha and 1 beta), and claudin proteins.[18-21]

In contrast to the later phases of cervical remodeling, major inflammatory events do not mediate the softening process. In Phase 1, only little changes in the distribution of macrophages or neutrophils are revealed.[7]

Cervical ripening (Phase 2)

Following softening, cervical ripening (Phase 2) occurs in the weeks or days preceding birth. The transition to Phase 2 is mediated by a decline in progesterone synthesis, increased cervical progesterone metabolism, and increased synthesis of estradiol and relaxin.[5] The results obtained by B. C. Timmons,[22] evident that cervical ripening requires downregulation of collagen assembly genes; increased synthesis of glycosaminoglycans that disrupt the matrix, such as hyaluronan; increased metabolism of progesterone; and changes in epithelial barrier properties. Cervical ripening is characterized by an increase in the content of hyaluronic acid, loosening of the collagen matrix, increased collagen solubility,[23,24] changes in the distribution of inflammatory cells, increased tissue growth and hydration, and loss of tensile strength.[25-28] Thus, this phase is characterized by maximal loss of tissue compliance and integrity.

During cervical ripening, alterations in collagen structure and packing are influenced by the composition of glycosaminoglycans (GAGs) in the extracellular matrix. Osmers and al.[29] showed that the clinical features of cervical ripening and dilatation were characterized by variation in the total glycosaminoglycan content and changes in the proportions of the different glycosaminoglycans (HA, dermatan sulfate, chondroitin sulfate, and heparan sulfate). The studies performed by Ruscheinsky et al.[30] suggest that HA has multiple, cell-specific functions in the cervix that may include modulation of tissue structure and integrity, epithelial cell migration and differentiation, and inflammatory responses. Increased hyaluronan synthase 2 expression and the subsequent increase in HA is a distinct feature of cervical ripening and dilation.[27] Proteoglycans containing sulfated GAG chains modulate collagen fibril size, spacing, and access to proteases.[31,32]

Several studies have suggested that normal cervical ripening may be a sterile inflammatory state characterized by an influx of immune cells into the cervix.[33-38] M. Mahendroo [16] and other authors [39,40] consider that immune cells are present but not activated during cervical ripening.

Cervical dilatation /labor (Phase 3)

According to Mendelson,[41] both term and preterm labor in humans and rodents are associated with an inflammatory response. If in preterm labor, intraamniotic infections may provide the stimulus for increased amniotic fluid interleukins and inflammatory cell migration,[42] at term, the stimulus for this inflammatory response is unknown.

Increasing evidence suggests that at term labor mechanical stretch [43,44] caused by the growing fetus, as well as hormonal signals produced by the developing fetus near term,[45-48] promote the production of chemokines leading to macrophage migration and up-regulation of inflammatory response pathways with the release of cytokines and activation of inflammatory transcription factors, such as NF-kB and AP-1, which also is activated by myometrial stretch.

Mendelson [41] postulates that the increased inflammatory response and NF-kappaB activation promote uterine contractility via 1) direct activation of contractile genes (e.g. COX-2,[49] oxytocin receptor,[50] and connexin 43)[51] and 2) impairment of the capacity of progesterone receptor to mediate uterine quiescence.

After the onset of regular uterine contractions, the ripened cervix is dilated sufficiently (Phase 3) to allow the passage of the full-term fetus through the birth canal. Given the short duration of the ripening and dilation phases, it is difficult to identify processes that distinguish these two overlapping phases of cervical remodeling. Just before birth, the final remodeling of the cervix is driven through the secretion of prostaglandins by the fetoplacental unit. Upon the increased secretion of cortisol by the fetal adrenals, prostaglandin synthase (PGHS)-2 gene expression in the placenta is up-regulated, resulting in increased production of PGE2 in the cervical region and subsequent matrix remodeling. [52]

According to Hassan et al.,[53] cervical dilatation in term labor is associated with a stereotypic gene expression pattern determined by microarray, which is characterized by overexpression of genes involved in neutrophil chemotaxis, apoptosis, extracellular matrix regulation, and steroid metabolism. The dilation phase has been well-studied in women due to the availability of cervix biopsies.

Postpartum repair (Phase 4)

Accumulating evidence suggests that human parturition represents an inflammatory process and the infiltrating leukocytes are a major source of pro-inflammatory mediators. Macrophages appear to play a more crucial role in the onset of parturition.[54] Macrophages account for around 20% of the decidual leukocyte population[55] and there is an influx of macrophages into the myometrium, fetal membranes, decidua, placenta, and cervix during spontaneous term labor [56, 57] and in preterm labor.[58]

Osman et al.[56] found that parturition was associated with a significant increase in IL-1beta, IL-6, and IL-8 mRNA expression in cervix and myometrium, IL-6 and IL-8 mRNA expression in chorio-decidua and IL-1beta and IL-8 mRNA expression in amnion. Histological analysis demonstrated that leukocytes (predominantly neutrophils and macrophages) infiltrate the uterine cervix coincident with the onset of labor. In a study performed by Young et al.,[37] such pro-inflammatory cytokines as IL-6, IL-8, and TNFα have been identified in the cervix during labor.

Characteristics of inflammation during the dilation phase are supported by the increased presence of inducible nitric oxide synthetase (iNOS) in the cervix stroma of women at term, whether or not in labor.[59, 50] Overall, these findings raise the possibility that some balance of immune cell products guides extracellular remodeling to promote softening, ripening, and the capability to dilate. [61]

During postpartum repair (Phase 4), the integrity and competence of cervical tissues are recovered to ensure a normal cervical function for subsequent pregnancies. Postpartum remodeling is characterized by decreased HA content, increased expression of genes involved in the assembly of mature collagen, synthesis of matrix proteins that promote a dense connective tissue, and inflammation.[22,30]

Matricellular proteins (SPARC, thrombospondin 1, thrombospondin 2, and tenascin C) modulating interactions between cells and the extracellular matrix[62] are expressed and regulated during cervical remodeling, but their specific function during postpartum repair remains to be elucidated.[63-66] A variety of factors, including metalloproteases, extracellular matrix proteins, and genes governing epithelial differentiation pathways, are all upregulated in postpartum,[22,39,67] as well as the expression of neutrophils, eosinophils, and both M1 and M2 macrophages.[5,16,37] The postpartum activation of M1 macrophages and neutrophils generate pro-inflammatory molecules that are important in matrix cleanup, whereas the alternatively activated M2 macrophages prevent overactivation of the inflammatory process and promote tissue repair.[5] Thus, the postpartum repair is characterized as a pro-inflammatory wound-healing response. [16,22,68,69]

Conclusion

Cervical remodeling is an active dynamic process that begins long before the onset of labor. The optimal course of the cervical ripening/remodeling processes is a prerequisite for successful vaginal delivery. Much more information about in vivo human tissue is necessary for a comprehensive understanding of the complex process of cervical remodeling. A better understanding of the fundamental biochemical and histophysiological processes occurring during cervical ripening is essential in the prevention of preterm labor and birth.

Competing Interests

The authors declare that they have no competing interests.

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BRIEF REVIEW

Falls in Elderly Patients with Stroke

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Abstract

The present review aims to identify the prevalence of falls, to describe the factors related to falls among elderly stroke patients, and to demonstrate the desired interventions to prevent falling among those patients. This review was conducted using PubMed, SCOPUS, Web of Science, and Medline to determine the incidence, risk factors, and favorable procedures to prevent and manage falls among elderly stroke patients. Falls lead to injuries, fractures, and impairments of the quality of life. Elderly stroke patients are more susceptible to falling than their healthy peers, which may result in greater restrictions on activity and mobility. Previous studies have documented the incredible prevalence of falls among the elderly who have suffered a stroke. Our study led to the conclusion that falls should be assessed to determine how to prevent and control them among elderly stroke patients. Physical therapy and rehabilitation modalities have a key role to play in improving the health status and prevent falling among those patients. (**International Journal of Biomedicine. 2020;10(4):330-333.**)

Key Words: stroke • falls • elderly • rehabilitation

Stroke is documented as the second commonest cause of death worldwide, after cardiac diseases, and they cause a high rate of disability, mortality, and morbidity worldwide.[1,2] Despite the worldwide decrease in the mortality rate in the last decade, the number of elderly people with stroke and fatal stroke increases each year, more in Asia than in America or Europe.[3]

Stroke commonly occurs among elderly populations, afflicting nearly 9% of the elderly between 65 and 79 years and around 15% of those older than 80 years.[4] By 2030, the number of stroke survivors among elderly populations may increase to approximately 10 million.[4,5]

Falls among stroke patients

Falls are considered as the most common health issue following a stroke, leading to high rates of mortality and morbidity.[6] The falling rate among stroke patients has ranged between 25% and 44%, particularly among those who are experiencing locomotor dysfunction.[7,8] Falling is a common complication among the aged population, increasing disability and dependence.[9] Approximately, 90% of the injuries among the elderly result from falling,[10] leading to a high economic burden for healthcare. Fractures commonly occur as a consequence of falls among the elderly, specifically hip fractures, which cause a potential disability and disturbance of health overall. In elderly stroke survivors, the risk of hip fracture is 4 times higher than among their peers in the general population.[10,11]

Characteristics of falling among elderly stroke patients

The falling features and circumstances for elderly and non-stroke patients have been assessed in early studies.[12,13] Nevertheless, studies that assess falling in post-stroke patients are limited. One previous study found that 50% of their studied sample of post-stroke patients had a history of falling related to disturbed balance and insufficient attention.[14] Another study reported that chronic stroke patients who suffered from falling had disturbed mobility and inability to perform their daily activities, with higher levels of depression and anxiety than stroke patients with no falling history.[15] However, it was documented that post-stroke patients commonly fall,[16] and risk factors of falling are numerous among stroke survivors.[14,15] Some studies have reported that the incidence of falling among older adult patients reaches 76%,[17,18] and other studies have reported that the incidence of falling among stroke patients is approximately 30%.[16,19] Other studies have reported that falling after a stroke results in injuries and that 20% of these injuries require a medical attention.[12,20]

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Despite a prior study that found similar characteristics among falling and non-falling stroke survivors in motor control and mobility, falling patients showed higher disabilities and comorbidities,[15] indicating that stroke associated with disabilities commonly contribute to falling. Also, another study concluded that stroke patients with orthostatic hypotension are commonly susceptible to falling.[21] Regarding the circumstances of falling, there is clearly a strong association between disability and falling risk. It is expected that falling can commonly take place in association with daily home activities, such as feeding, washing, showering, and toileting. In addition to falling related to daily home activities, patients commonly fall because of slips or trips. Geriatric literature has reported a high prevalence of slips and trips on such small obstructions as sidewalk cracks, carpets, curbs, cords, and roots of trees.[12,22] Also, stroke survivors may slip or trip on ice or wet surfaces because of diminished vision or other sensory abilities.

Falling history has documented that falls are likely associated with physical and mental impairments, including carelessness in some home details, such as alcohol drinking, poor quality of sleep, tying shoes, and the use of assistive devices. Therefore, the prevention of falling after a stroke should not concentrate only on improving range of motion and muscle strengthening but also on modifying associated risk factors of falling.

Risk factors for falling among elderly stroke patients

Generally, independent risk factors for falls among the elderly include muscle weakness, recurrent falling, psychological medications, visual or other sensory deficiency, disturbed gait, walking deficits, physical restrictions, anxiety, dizziness, depression, cognitive dysfunction, orthostatic hypotension, incontinence, diabetes, female gender, receiving poly medications, and being older than 80 years.[23]

Recently, a systematic review demonstrated that among the elderly population some risk factors have a strong relationship to falling, such as falling history, disturbed gait, using walking aids, neurological diseases, vertigo, and use of antiepileptic medications.[24] Also, an observational study found that lesions of the white-matter area of the brain are likely a risk factor predictor for falling.[25] Further, it has been documented that the falling risks are directly related to the pain severity of musculoskeletal disorders, the number of affected joints, and amount of interference with daily living activities.[26] Orthostatic hypotension is commonly prevalent among the elderly, leading to gait impairment and falling.[27] Moreover, another study on four elderly subjects documented that obstructive sleep apnea leads to falling-related injuries.[28]

Regarding falling among elderly stroke patients, in addition to the previous risk factors, a recent study found a positive correlation between the prevalence of falling and indoor tripping hazards with no differences between stroke patients and non-stroke peers, suggesting that for stroke patients there is a greater risk of falling outdoors than indoors. [29] Stroke patients have a higher susceptibility to falling than matched controls,[30,31] and the risk of falls increases with high physical disabilities.[32] Both indoor and outdoor environments may raise the probability of falls among stroke survivors, specifically those experiencing physical limitations.[33,34] The majority of outdoor predictors for falling are sidewalks and sidewalk obstructions;[35] however, the indoor predictors are associated with tripping risks, such as poor light and loose carpets.[36] Anxiety and fear of falling are additional risk factors observed among those who fall outdoors.[37] Fear of falling may be combined with social factors that prevent the survivor from asking family members or neighbors to assist with walking.[38]

Prevention of falling among elderly stroke patients

Previous studies have shown several successful treatment modalities for preventing falls and associated risk factors.[39-41] An effortless and easy modality having a highly beneficial influence is a home assistant to pick up anything that presents a risk of tripping and to clean up clutter and clear walking pathways at home. Generally, treatment modalities for preventing falling among the elderly are classified into particular or multi-section treatment. The particular, evidence-based treatment for preventing falls among the elderly include home evaluation and adjustment for those at high-risk, adherence to exercise training such as gait training, strengthening, balance, and stretching exercises, and receiving more than 700IU of vitamin D supplements per day.[42-44] However, the multi-section treatment shows beneficial effects to prevent falling among the elderly, including reviewing and reducing psychological medications,[42] assessing and treating orthostatic hypotension,[23] rapid cataract surgery for an impacted eye,[42] wearing multifocal glasses when outdoors,[45] using anti-slipping shoes outdoors during winter,[46] and cardiac pacing in cardiac inhibitory carotid sinus syndrome.[47] Multi-section treatment is strongly recommended because it assesses individualized risk factors for falling and planning the intervention program for reducing that risk.[42]

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Competing Interests

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Cardiology

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The Effectiveness of Triple Fixed-Dose Combination Therapy in the Management of Uncontrolled Arterial Hypertension

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Abstract

The aim of our study was to identify patients with uncontrolled hypertension who were resistant to previous antihypertensive therapy and to assess the clinical efficacy of the fixed-dose triple combination therapy during 6 months.

Methods and Results: The study included 156 patients with uncontrolled hypertension. The mean age of patients was 57.81±10.8 years; the average duration of arterial hypertension was 8.87±5.34 years. According to the questionnaire data, all patients received dual or triple free-combination antihypertensive therapy, but did not reach the target blood pressure level. After the screening stage, all patients were discontinued from previous therapy and assigned to the fixed-dose triple combination therapy. Patients initially taking angiotensin-converting enzyme inhibitors (n=96/61.5%) were switched to single-pill triple combination of perindopril, indapamide and amlodipine (Per/Ind/Aml [5mg/1.25mg/5mg]). Patients initially taking angiotensin receptor blockers (n=60/38.5%) were switched to single-pill triple combination of telmisartan, hydrochlorothiazide and amlodipine (Tel/HCTZ/Aml [40mg/12.5mg/5mg]). Since the purpose of our study was not to compare the effectiveness of 2-drug regimens based on angiotensin-converting enzyme inhibitor or angiotensin receptor blocker, we combined all patients into one group and monitored the dynamics after 3 months and 6 months of treatment.

The primary target level for systolic blood pressuer (SBP) and diastolic blood pressure (DBP) was <140 mmHg and <90 mmHg, respectively. With good tolerability of therapy, we aimed for the recommended target level of SBP/DBP (<130 mmHg/<80 mmHg).

All patients underwent the following examinations: assessment of traditional risk factors, physical examination, clinical and biochemical laboratory methods, 12-lead ECG, echocardiography, pulse contour analysis, and 24-hour ambulatory blood pressure monitoring.

The results of our study showed that the fixed-dose triple combination therapy at the indicated initial doses in patients with uncontrolled hypertension made it possible to achieve the primary target blood pressure level in 73.7% of patients after 3 months of treatment. A further increase in single-pill triple combination doses made it possible to increase the number of patients (after 6 months of treatment) who achieved the primary goal to 92.3%, and the recommended goal in 82.4% of patients. After 6 months of treatment, against the background of selected doses of single-pill triple combination, 7.7% of patients were diagnosed with treatment-resistant hypertension.

The echocardiography data showed a significant decrease in left ventricular mass index by -9.7±7.3% after the 3-month treatment. After 6 months of treatment, there was a further decrease in left ventricular mass index up to -13.5±10.1%, while 34% of patients had no left ventricular hypertrophy, according to left ventricular mass index. After the 6-month treatment, left ventricular hypertrophy remained in 65.4% of patients versus 89.7% before starting therapy. The 6-month dynamics of the left ventricular diastolic dysfunction indicator also slightly improved; the left ventricular diastolic dysfunction was not determined in 25% patients.

A retrospective analysis of the hemodynamic data of patients before treatment revealed significantly high indicators for the office SBP, DBP, and pulse pressure, the central SBP, pulse wave velocity, the average daily, daytime and nighttime SBP, and a higher left ventricular mass index and left ventricular diastolic dysfunction in patients with uncontrolled hypertension than in patients who achieved target blood pressure levels after 3 months of therapy

Conclusion: Markers of uncontrolled hypertension are high SBP, DBP, and increased pulse wave velocity, which requires the maximum doses of a full-dose single-pill triple combination of antihypertensive drugs (Per/Ind/Aml or Tel/HCTZ/Aml), without wasting time on titration of drug doses. (**International Journal of Biomedicine. 2020;10(4):334-341.**)

Key Words: uncontrolled hypertension • antihypertensive drug • fixed-dose triple combination • treatment-resistant hypertension

Abbreviations

AH, arterial hypertension; **ABPM**, ambulatory blood pressure monitoring; **AHD**, antihypertensive drug; **AHT**, antihypertensive therapy; **ACEIs**, angiotensin-converting enzyme inhibitors; **ARBs**, angiotensin receptor blockers; **BP**, blood pressure; **CCBs**, calcium channel blockers; **DBP**, diastolic BP; **DBPc**, central DBP; **FDTC,** fixed-dose triple combination; **LVMI**, left ventricular mass index; **LVH**, left ventricular hypertrophy; **LVDD**, LV diastolic dysfunction; **PWV**, pulse wave velocity; **PP**, pulse pressure; **PPc**, central PP; **RAAS**, renin-angiotensin-aldosterone system; **SBP**, systolic BP; **SBPc**, central SBP; **SPTC**, single-pill triple combination; **TRH**, treatment-resistant hypertension; **T2DM**, type 2 diabetes mellitus; **UCH,** uncontrolled hypertension.

Introduction

Arterial hypertension (AH) remains an urgent problem in clinical medicine, being a serious risk factor for cardiovascular complications. AH has a widespread prevalence among the world's adult population. The STEPS study, conducted in Uzbekistan in 2014, showed a high prevalence (30.8%) of hypertension among adults 18-64 years old. However, it should be noted that this study did not cover patients over age 65, among whom, according to epidemiological data, the prevalence of hypertension increases 2 or more times, compared to younger people.[1,2]

Despite the widespread clinical introduction of combination antihypertensive therapy (AHT), in some patients blood pressure (BP) cannot be controlled, and treatment-resistant hypertension (TRH) is revealed. Predictors of insufficient BP control are old age (over age 75), left ventricular hypertrophy (LVH), obesity, SBP>160 mmHg, type 2 diabetes mellitus (T2DM), increased consumption of salts, and obstructive sleep apnea. Currently, uncontrolled hypertension (UCH) is increasing throughout the world, which is associated with a decrease in target BP levels, with an increase in population life expectancy, a more frequent incidence of co-morbid pathology, and insufficient control of cardiovascular risk factors.[3] Of no small importance is the wrong choice or dosage of antihypertensive drugs (AHDs), the lack of synergy of action when using a combination of drugs or irrational AHD combinations, and problems associated with adherence to treatment. These data are worrisome because there is strong evidence of the practical importance of lowering BP to the target level, which results in a reduction in the risk of myocardial infarction by 20%-25%, heart failure by 50% and stroke by 35%-40%.[4] One of the forms of UCH is TRH, in which, despite the long-term use of ≥3 AHDs with different mechanisms of action, including diuretics, excessive sodium reabsorption remains in the distal segments of the patient's nephron, which is caused by increased aldosterone activity and hyperactivity of the sympathetic nervous system.[5]

According to current recommendations, the first step in choosing AHT is a dual-combination of AHDs, one of which is an RAAS blocker, and the other a calcium channel blocker (CCB) or a diuretic, preferably in a single pill. The choice of a dual-combination AHT at the start of hypertension treatment provides complete control of BP and protection of target organs. In case there is resistance to the dual-combination AHT, it is recommended to switch to a triple combination AHT, consisting of angiotensin-converting enzyme inhibitor (ACEI)/angiotensin receptor blocker (ARB), CCB and diuretics, also preferably in a single pill, in order to increase patient adherence to therapy. According to epidemiological studies, 10%-12% of patients with AH are resistant to the fixed-dose triple combination (FDTC) therapy, which indicates the presence of TRH and requires the addition of the fourth AHD. As a fourth drug, the use of spironolactone, beta-blockers, alpha-blockers or other AHD is recommended.[6]

The aim of our study was to identify patients with UCH who were resistant to previous AHT and to assess the clinical efficacy of the FDTC therapy during 6 months.

Materials and Methods

The study was carried out in compliance with Ethical Principles for Medical Research Involving Human Subjects, Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964, and amended by the 64th WMA General Assembly, Fortaleza, Brazil, October 2013 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.

The study included 156 patients with UCH. The mean age of patients was 57.81±10.8 years; the average duration of AH was 8.87±5.34 years.

Exclusion criteria were symptomatic hypertension; valvular heart disease, acute coronary syndrome, chronic heart failure (NYHA FC>III), cardiac arrhythmia, history of stroke and myocardial infarction within previous 12 months, occlusive peripheral arterial disease, renal impairment, severe co-morbidities, orthostatic hypotension.

According to the questionnaire data, all patients received dual or triple free-combination AHT, but did not reach the target BP level. After the screening stage, all patients were discontinued from previous therapy and assigned to the FDTC therapy. Patients initially taking ACEIs (n=96/65.8%) were switched to single-pill triple combination (SPTC) of perindopril, indapamide and amlodipine (Per/Ind/Aml [5mg/1.25mg/5mg]). Patients initially taking ARBs (n=60/34.2%) were switched to SPTC of telmisartan, hydrochlorothiazide and amlodipine (Tel/HCTZ/Aml [40mg/12.5mg/5mg]). Since the purpose of our study was not to compare the effectiveness of 2-drug regimens based on ACEI or ARB, we combined all patients into one group and monitored the dynamics after 3 months and 6 months of treatment.

The effectiveness of therapy was assessed by achieving the target BP level according to 2018 ESH/ESH Guidelines for the management of AH. The primary target level for SBP and DBP was <140 mmHg and <90 mmHg, respectively. With good tolerability of therapy, we aimed for the recommended target level of SBP/DBP (<130 mmHg/<80 mmHg).

All patients underwent the following examinations: assessment of traditional risk factors, physical examination,

clinical and biochemical laboratory methods, 12-lead ECG, echocardiography, and 24-hour ABPM. Office BP was measured using a mercury sphygmomanometer, according to Korotkov's method. BP was measured 3 times, and the means of these measurements were used in the analyses. The 24-hour ABPM was performed using a BR-102 plus (SCHILLER, Switzerland).

The pulse contour analysis was carried out using the SphygmoCor device (AtCor Medical, Australia), which obtains peripheral arterial pressure waveforms by applying an arterial applanation tonometer to the wrist. The tonometer partially compresses the radial artery and records a pressure wave over several cardiac cycles. This pressure wave is calibrated to brachial cuff blood pressure measurements. The averaged peripheral waveform is then converted to an ascending aortic waveform using a generalised mathematical transfer function. In addition, such indicators as the central SBP (SBPc), central DBP (DBPc), central PP (PPc), and PWV were taken into account.

Echocardiography was carried out according to the recommendations of the American Society of Echocardiography[7] in M- and B-modes using Philips EnVisor C Ultrasound Machine (the Netherlands). The following parameters were measured and calculated: IVST, PWT, LVEDD, LVESD, EF, LVEVD, LVESV, and LVM (LVM was calculated using the formula R. Devereux.[8] LVM was indexed to body surface area (LVMI). Left ventricular hypertrophy (LVH) was defined as LVMI of >95 g/m^2 (for women) and >115 g/m^2 (for men).[6]

The ratio of peak early filling velocity to peak atria1 filling velocity (PE/PA) was calculated. The isovolumic relaxation phase (IRP) was also measured.

Statistical analysis was performed using the statistical software «Statistica». (v6.0, StatSoft, USA). Baseline characteristics were summarized as frequencies and percentages for categorical variables and as mean± standard deviation (SD) for continuous variables. Student's paired t-test was used to compare two groups for data with normal distribution. Group comparisons with respect to categorical variables were performed using chi-square test. A probability value of $P<0.05$ was considered statistically significant.

Results

Among 156 UCH patients, increased body weight and obesity were found in 147(94.2%) patients, smoking in 51(32.7%), T2DM managed without insulin in 21(13.5%), coronary heart disease (FC I-II) in 55(35.2%), and dyslipidemia in 129(82.7%) patients. LVH and LVDD were detected in 140(89.7%) and 128(82.1%) patients, respectively (Table 1).

Thus, all UCH patients had a high or very high risk of cardiovascular complications. The targeted lifestyle modifications were recommended for all patients. For patients with dyslipidemia, atorvastatin was recommended at a dose of 20 mg/day. Patients with a history of coronary heart disease and T2DM received acetylsalicylic acid.

After 3 months of the FDTC therapy at the indicated initial doses, we noted in the group as a whole a significant decrease in the office SBP by -20.1±7.52%, DBP by -17.7±8.67%, as well as a significant decrease in the central SBP and DBP and an improvement in the 24-hour ABP profile (Table 2). The primary target level of SBP/DBP was reached in 115(73.7%) patients. Of these, 82(52.5%) reached the recommended target SBP/DBP level after 3 months of therapy. At the same time, 41(26.3%) patients did not reach the primary target BP level at the initial doses of SPTC, which required an increase in the dose of drugs to the maximum tolerable: Per/Ind/Aml – 10mg/2.5mg/10mg, and Tel/HCTZ/Aml – 80mg/25mg/10mg, respectively. Patients given a high-dose combination of Per/Ind/Aml (10mg/2.5mg/10mg) took 1 pill once a day. Due to the lack of a high-dose combination of Tel/HCTZ/Aml in the pharmacological market, 2 pills of 40mg/12.5mg/5mg once a day were prescribed. Patients who achieved the primary target SBP/DBP levels continued therapy with the previous doses of FDTC of AHDs.

Table 1.
Clinical characteristics of patients with uncontrolled hypertension

Variable	General group (n=156)
Age, years	57.81±10.8
Average duration of AH, years	8.87±5.34
BMI, kg/m^2	31.7±4.58
Waist circumference, cm	98.87±9.74
Gender: Male Female	 88 (56.4%) 68 (43.6%)
AH: Grade 1 Grade 2 Grade 3	46 (29.5%) 101 (64.7%) 9 (5.8%)
Normal body weight, % Increased body weight, % Obesity, %	9 (5.8%) 41 (26.3%) 106 (67.9%)
LDL cholesterol >100 mg/dl	129 (82.7%)
CHD (FC I-II)	55 (35.2%)
T2DM	21 (13.5%)
Smoking	51 (32.7%)
LVH	140 (89.7%)
LVDD	128 (82.1%)

After 6 months of therapy, a further decrease in the levels of SBP and DBP was observed, and the primary target BP level was achieved in another 29 patients. In total, against the background of the 6-month FDTC therapy at the indicated initial doses, the primary target BP level was achieved in 144 (92.3%) patients and the recommended target BP level in 128(82.4%) patients. At the same time, SBP decreased by -25.15±5.73% and DBP by -22.8±6.44%. By the end of the 6-month treatment, 12 patients who received full-dose SPTC of AHDs were characterized as TRH patients (Table 2).

According to ABPM data, the target BP levels were achieved in the average daily and daytime SBP and DBP.

Table 2.

Clinical efficacy of the FDTC therapy at the initial doses in the general group of patients with AH

Variable	Before treatment (1)	After 3 months of the FDTC therapy (2)	P_{1-2}	After 6 months of the FDTC therapy (3)	P_{1-3}
SBP, mmHg	163.26±16.97	128.7±9.65	0.0001	123.17±7.67	0.0001
DBP, mmHg	99.56±8.42	81.48±7.37	0.0001	77.53±5.26	0.0001
PP, mmHg	63.69±14.06	47.32±7.22	0.0001	45.63±5.75	0.0001
SBPc, mmHg	156.37±23.3	142.0±17.7	0.0001	130.86±15.5	0.0001
DBPc, mmHg	92.64±15.02	86.1±13.6	0.0001	85.34±11.4	0.0001
PPc, mmHg	63.3±21.54	55.7±16.52	0.035	46.0±14.08	0.001
PWV, m/sec	10.62±2.36	9.97±1.99	0.028	9.4±1.8	0.02
Average 24-h SBP, mmHg	142.4±15.15	129.7±14.46	0.0001	126.87±12.07	0.0001
Average 24-h DBP, mmHg	86.86±11.19	80.13±10.88	0.0001	78.87±7.02	0.0001
Average daytime SBP, mmHg	144.0±16.26	131.7±14.99	0.0001	129.9±12.6	0.0001
Average daytime DBP, mmHg	88.98±11.98	82.42±11.12	0.0001	81.31±9.0	0.0001
Average nighttime SBP, mmHg	134.8±16.98	124.4±14.66	0.0001	121.44±10.8	0.0001
Average nighttime DBP, mmHg	80.76±12.46	74.29±10.8	0.0001	74.56±6.65	0.0001
LVM, g	298.36±58.25	255.84±68.36	0.0001	247.03±69.7	0.0001
LVMI, g/m^2	148.54±30.2	128.7±29.7	0.0001	125.5±30.85	0.0001
E/A	0.81±0.25	0.84±0.22	0.26	0.9±0.24	0.001

Table 3.

Clinical and hemodynamic characteristics of patients with controlled and uncontrolled hypertension after 3 months of the FDTC therapy

Параметры	Patients with controlled hypertension, n=115	*P*-value	Patients with uncontrolled hypertension, n=41
SBP, mmHg	158.7±15.23	0.0001	173.87±16.34
DBP, mmHg	98.44±9.16	0.053	103.1±7.2
PP, mmHg	60.71±11.58	0.0001	71.88±14.62
SBPc, mmHg	149.5±22.54	0.0001	173.78±32.67
DBPc, mmHg	91.95±15.23	0.51	95.0±15.88
PPc, mmHg	57.24±18.1	0.0001	77.55±32.87
PWV, m/sec	10.2±2.24	0.003	12.12±2.88
Average 24- h SBP, mmHg	139.5±13.66	0.002	151.4±18.2
Average 24- h DBP, mmHg	85.54±10.4	0.346	88.33±13.06
Average daytime SBP, mmHg	140.6±14.28	0.003	152.2±18.24
Average daytime DBP, mmHg	87.49±10.97	0.31	90.33±14.0
Average nighttime SBP, mmHg	132.5±16.36	0.006	144.17±18.56
Average nighttime DBP, mmHg	80.25±13.08	0.77	81.85±11.54
LVM, g	275.6±74.6	0.044	303.2±75.5
LVMI, g/m^2	138.27±30.7	0.052	149.3±31.9
E/A	0.83±0.25	0.25	0.78±0.2

After 6 months of therapy, the average nighttime SBP and DBP decreased slightly, compared to 3-month data, but the target values were not achieved.

The echocardiography data showed a significant decrease in LVMI by -9.7±7.3% after the 3-month treatment. After 6 months of treatment, there was a further decrease in LVMI up to -13.5±10.1%, while 34% of patients had no LVH, according to LVMI. After the 6-month treatment, LVH remained in 65.4% of patients versus 89.7% before starting therapy. The 6-month dynamics of the LVDD indicator also slightly improved; LVDD was not determined in 25% of patients.

To identify predictors of insufficient BP control, we compared the baseline data of patients who achieved and did not achieve the primary target BP level. After 3 months of treatment with SPTC in the initial doses in groups with controlled and uncontrolled hypertension, the degree of SBP reduction was -21.2±6.8% and -16.54±7.8% (P=0.008), respectively, and for DBP -18.3±7.5% and -12.62±7.66% (P=0.0001), respectively. In the group of patients with UCH versus controlled hypertension, the number of patients with T2DM was significantly higher (24.4% and 12.6%, respectively, [P<0.05]).

A retrospective analysis of the hemodynamic data of patients before treatment revealed significantly high indicators for the office SBP, DBP, and PP, the central SBP, PWV, the average daily, daytime and nighttime SBP, and a higher LVMI and LVDD in patients with uncontrolled hypertension than in patients who achieved target blood pressure levels after 3 months of therapy (Table 3). The results obtained indicate the possibility of using a full-dose SPTC (Per/Ind/Aml and Tel/HCTZ/Aml) at the start of treatment in patients with uncontrolled hypertension.

Discussion

Treatment-resistant hypertension affects between 3% and 30% of hypertensive patients, and its presence is associated with increased cardiovascular morbidity and mortality.[9] Among the reasons for poor hypertension control, the most important are inappropriate treatment regimens (no combination therapy, inadequate doses, or inappropriate combinations) and poor adherence to treatment. As is known, PWV >10 m/s, 24-h PP≥63 mmHg, or central PP>55 mmHg suggests vascular remodelling. The absence of increased arterial stiffness suggests the presence of pseudo-resistance that suggests to search for poor treatment adherence, life-style factors known to increase arterial BP, and drugs interfering with antihypertensive therapy.[9]

A group of Russian and Norwegian scientists examined uncontrolled hypertension and differences in treatment regimens between a high-risk country, Russia, and a low-risk one, Norway, to gain a better understanding of the underlying factors.[10] Population-based survey data on 40- 69-year-olds with hypertension were obtained from Know Your Heart Study (KYH, n=2284) performed in Russia (2015-2018), and the seventh wave of The Tromsø Study (Tromsø 7, n=5939) performed in Norway (2015-2016). Among all those with hypertension, regardless of treatment status, control of BP was achieved in 22% of men (KYH and Tromsø 7), while among women it was 33% in Tromsø 7 and 43% in KYH. When the analysis was limited to those on treatment for hypertension, the percentage of uncontrolled hypertension was higher in KYH (47.8%, CI 95 44.6-50.9%) than Tromsø 7 (38.2, 36.1-40.5%). The corresponding figures for apparent treatment-resistant hypertension were 9.8% (8.2%-11.7%) and 5.7% (4.8-6.8%), respectively. Antihypertensive monotherapies were more common than combinations and used by 58% in Tromsø 7 and 44% in KYH. The authors concluded that the factors associated with untreated hypertension overlap with known correlates of non-adherence to treatment and non-attendance at health checks. In contrast, apparent treatment-resistant hypertension was characterized by obesity and underlying comorbidities, potentially complicating treatment.

International guidelines for the treatment of arterial hypertension indicate that 2 regimens of therapy, depending on the initial cardiovascular risk , are capable of achieving the target blood pressure level: monotherapy and combination therapy.[6] For hypertensive patients with a high cardiovascular risk, it is recommended to start combined drug treatment and a rapid dose increase. In the ACCOMPLISH study, 74% of hypertensive patients with a high cardiovascular risk had already received 2 or more antihypertensive drugs at baseline prior to enrollment in the study.[11]

According to National Clinical Guideline Centre (UK), 1 in 5 hypertensive patients is on a combination of 3 or more antihypertensive drugs. In this case, the most commonly used combination consists of diuretics, ACEIs and CCBs, compared to other three-component combinations.[12]

According to the Health Survey for England (HSE), the most prescribed drugs in England (2011) were RAAS blockers (53%), CCB (20%), then diuretics (15%), beta-blockers (11%), and other drugs (1%).At the same time, for triple-antihypertensive drug combinations, a combination of diuretics, ACEIs/ARBs and CCBs was more often prescribed than other triple combinations.[13]

According to a statement by the American Society of Hypertension and the International Society of Hypertension (2013), a triple combination of ACEI or ARB/CCB/diuretics in full or maximally tolerated doses is recommended for treatment of uncontrolled hypertension.[14] The same approach to the choice of a triple combination of antihypertensive drugs is indicated in the 2018 ESH/ESH Guidelines with an emphasis on the choice of fixed-dose triple combination of antihypertensive drugs (ACEI or ARB/CCB/diuretic in a single pill).[6]

As shown in the ADVANCE trial, in hypertensive patients with type 2 diabetes mellitus, the addition of CCBs against the background of treatment with indapamide and perindopril doubled both the antihypertensive efficacy and the effectiveness in reducing cardiovascular mortality from 14% to 28% in high-risk hypertensive patients. Even the use of a low-dose, triple combination of antihypertensive drugs made it possible to achieve the target blood pressure level, and a triple combination of ACEI/CCB/diuretics in full or maximally tolerated doses normalized blood pressure in patients considered resistant to antihypertensive therapy.[15]

The PIANIST study showed that the single-pill triple combination containing Per/Ind/Aml was effectively and safely administered to a large population of high- and very-high-risk hypertensive patients who had not reached target office BP values with previous treatment.[16]

In a study performed by Nedogoda et al.,[17] AH patients were randomly assigned to: single-pill triple combination of Per(5mg)/Ind(1.25mg)/Aml(5mg) or a dual-pill combination of Per(5mg)/Ind(1.25mg) + Aml(5mg) once daily for 12 weeks. The primary endpoint was a change in office supine SBP and DBP from baseline to Week 12. At Week 12, both triple-therapy regimens were associated with clinically significant reductions in SBP, compared with baseline (-21.5±11.7 mmHg and -20.0±12.9 mmHg, respectively) Reductions in office supine DBP were also clinically significant (-15.3±7.8 mmHg and -14.8±9.0 mmHg, respectively). The majority of patients were treatment responders at Week 4 (89.2 and 82.9%, respectively) and had achieved blood pressure control (87.8 vs. 78.6%, respectively), which was maintained until Week 12 in both treatment groups. Both treatments were well tolerated with no between-group differences.

A study conducted by Mazza et al.[18] compared the efficacy of fixed-dose triple combination of antihypertensive drugs with that of a free combination of 3 antihypertensives in patients with uncontrolled hypertension. Ninety-two patients with uncontrolled hypertension previously treated with an RAAS inhibitor plus HCTZ were switched to once-daily fixed-dose triple combination therapy with Per/Ind/Aml(5-10/1.25-2.5/5-10 mg). Significant reductions in ambulatory 24-h, daytime and nighttime SBP, and PP were found in the fixed-dose triple combination group, relative to reductions seen with free-combination therapy, after the first month only of follow-up. Target blood pressure values (mean 24-h ambulatory SBP/DBP <130/80 mmHg) were reached by more recipients of fixed-dose triple combination than free-combination therapy (64.8% vs. 46.9%, $P<0.05$) at Month 4 of follow-up.

Of the fixed-dose triple combination, including ARBs, combinations of valsartan/olmesartan with HCTZ and amlodipine have been studied, which were approved by the European Medicines Agency in 2009. Stafylas et al.[19] compared the cost-utility of the first available single-pill triple combination containing valsartan (Val), Aml and HCTZ, with each of the same components in dual combinations in patients with moderate to severe hypertension. A Markov model with eight health states was constructed. The short-term effect of antihypertensive therapy on BP was extrapolated through the Hellenic SCORE and Framingham risk equations, estimating the long-term survival and quality-adjusted life-years (QALYs) saved. The incremental cost-effectiveness ratio of the triple combination versus Val/Aml and Aml/HCTZ was far lower than the Greek GDP per capita and really close for Val/HCTZ, suggesting the Val/Aml/HCTZ combination to be cost-effective. The probability that the single-pill triple combination is cost-effective was more than 90%. The authors concluded that a single-pill Val/Aml/HCTZ therapy is a cost-effective antihypertensive choice for the treatment of moderate to severe hypertension, compared to its dual components.

Telmisartan, like valsartan, is the most studied ARB in high-risk hypertensive patients with metabolic syndrome, type 2 diabetes mellitus, and nephropathy. Telmisartan is the most long-acting ARB available today. It is of interest to compare the effectiveness of this drug in lowering blood pressure with valsartan. Takagi et al.[20] performed an updated meta-analysis of randomized controlled trials of telmisartan versus losartan therapy for reduction of ambulatory blood pressure in patients with arterial hypertension. The meta-analysis included a total of 2409 patients with hypertension. Pooled analysis suggested significant reductions in all of 24-h (mean differences of SBP/DBP, -2.09/-1.57 mmHg), last 6-h (-2.96/-2.15 mmHg), morning (-2.71/-2.37 mmHg), daytime (-1.74/-1.73 mmHg) and nighttime blood pressure (-2.70/-2.08 mmHg) among patients randomized to telmisartan versus losartan therapy. Thus, a statistically significant result favored telmisartan over losartan therapy.

Thus, according to the data of randomized clinical trials, the selected single-pill triple combinations based on the RAAS blockers, diuretics, and CCB were not inferior to each other and had high clinical efficacy. The selected combinations of Per/Ind/Aml and Tel/HCTZ/Aml are 2 of the desired rational combinations: Aml and diuretics have natriuretic and vasodilating actions, and Per/Tel blocks RAAS hyperactivity. Thus, the antihypertensive and organ-protective efficacy of drugs is potentiated, resulting in a decrease in morbidity and cardiovascular mortality. Complex treatment of patients with non-drug correction of risk factors and single-pill triple combination therapy makes it possible to reduce the number of patients with uncontrolled hypertension in whom the previous free-combination therapy with 2 and even 3 antihypertensive drugs was not effective.

The results of our study showed that the fixed-dose triple combination therapy at the indicated initial doses in patients with uncontrolled hypertension made it possible to achieve the primary target BP level in 73.7% of patients after 3 months of treatment. A further increase in single-pill triple combination doses made it possible to increase the number of patients who achieved the primary goal to 92.3%, and the recommended goal in 82.4% of patients.

As shown by a comparative analysis of 3-month treatment, according to baseline data, 41 patients who did not reach the primary target BP level had significantly higher levels of the office SBP, DBP, and PP, central SBP, PWV, the average daily, daytime and nighttime SBP than patients (n=115) who achieved target BP levels. An increase in the dose of drugs to the maximum tolerable (Per/Ind/Aml [10mg/2.5mg/10mg] and Tel/HCTZ/Aml [80mg/25mg/10mg], respectively), after 6 months of therapy, led to a further decrease in the levels of SBP and DBP, with the achievement of the primary target BP level in another 29 patients. The results obtained indicate the possibility of using a full-dose single-pill triple combination (Per/Ind/Aml and Tel/HCTZ/Aml) at the start of treatment in patients with uncontrolled hypertension. After 6 months of therapy, 12 patients who received full-dose single-pill triple combination treatment were characterized as patients with treatment-resistant hypertension, which required the addition of the fourth antihypertensive drug according to the recommendations.[6]

Conclusion

Despite the fact that our study was limited in the number of patients with uncontrolled hypertension and the duration of follow-up, a 6-month fixed-dose triple combination therapy (Per/Ind/Aml and Tel/HCTZ/Aml) in this study showed a high antihypertensive and cardio-protective efficacy, and a reliably positive effect on the daily blood pressure profile, central blood pressure, with a decrease in PWV and LVMI. Against the background of selected doses of single-pill triple combination, 92.3% of patients with uncontrolled hypertension reached the primary target blood pressure level (<140/90 mmHg) and 82.4% of patients reached the recommended target blood pressure level (<130/80 mmHg); 7.7% of patients were diagnosed with treatment-resistant hypertension. Markers of uncontrolled hypertension are high SBP, DBP, and increased PWV, which requires the maximum doses of a full-dose single-pill triple combination of antihypertensive drugs, without wasting time on titration of drug doses.

Competing Interests

The authors declare that they have no competing interests.

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sST2 Protein Serum Levels in Patients with Chronic Heart Failure

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Abstract

The purpose of this study was to determine the blood concentration of sST2 and evaluate the correlations between sST2 and the clinical, instrumental, and echocardiographic characteristics of patients with CHFrEF (chronic heart failure with reduced ejection fraction) and CHFpEF (chronic heart failure with preserved ejection fraction), as well as to evaluate the sST2 informativeness in comparison with NT-proBNP.

The study involved 160 patients diagnosed with CHF (86 men and 74 women, mean age of 72.4±8.7 years). According to the LVEF value, the patients were divided into two groups: CHFpEF (EF≥50%) was recorded in 69 people, CHFrEF (EF<50%) - in 91 people. The functional class (FC) of CHF was determined according to the NYHA classification (1994). NT-proBNP and hs-CRP levels had a stronger positive association with sST2 levels in CHFrEF patients than in CHFpEF patients. The results obtained (regardless of the EF) indicated a statistically significant direct correlation between the levels of sST2 and LAVI. The weak direct correlation between sST2 and NYHA FC, in contrast to the direct strong correlation between NT-proBNP and sST2, indicates that sST2, which is quite important both for determining the current state of the patient and for predicting the course of the disease, still cannot be used as a single and universal indicator. In this regard, further comprehensive study of the diagnostic and prognostic capabilities of the sST2 and NT-proBNP biomarkers in CHF patients with different EF is required. (**International Journal of Biomedicine. 2020;10(4):342-346.**)

Key Words: sST2 • NT-proBNP • hs-CRP • CHFrEF • CHFpEF

Abbreviations

CHF, chronic heart failure; **EF**, ejection fraction; **CHFrEF**, CHF with reduced EF, **CHFpEF**, CHF with preserved EF; **IL1RL1**, interleukin 1 receptor like 1; **LAVI**, left atrial volume index; **LVMI**, left ventricular mass index; **LVEF**, left ventricular EF; **TAPSE**, tricuspid annular plane systolic excursion; **6MWT**, the 6-minute walking test; **E**, transmitral early peak velocity; ***e'***, early diastolic mitral annulus velocity; **NT-proBNP**, N-terminal pro-brain natriuretic peptide; **hs-CRP**, high-sensitivity C-reactive protein.

Introduction

Chronic heart failure (CHF) is a rapidly growing public health issue associated with increased morbidity and mortality. Modern understanding of the CHF pathogenesis indicates the heterogeneity of the processes occurring in the myocardium, depending on LVEF.[1-3] In the literature, CHFrEF is discussed in detail.[4] Nevertheless, about half of patients with CHF have LVEF>50%, which corresponds to CHFpEF, and morbidity and mortality rates are comparable for both groups.[5]

It is believed that endogenous low-level inflammation, cellular hypertrophy, and an increase in myocardial stiffness (due to extracellular reorganization) are the determining processes in the formation of CHFpEF. Essentially, CHFpFV is a process of proliferation of connective tissue and disorders of cell metabolism.[6] Various pathogenetic cascades imply the likelihood that various biologically active substances have participated in their implementation. Many biomarkers are being actively studied for their use as methods for diagnosing

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and monitoring the stability of CHF patients.[7] In particular, it is NT-proBNP, hs-CRP, and the less studied ST2 protein.

The Il1rl1 gene product, also having the alias ST2, is a member of the interleukin 1 receptor superfamily.[8,9] For the first time, it has become known as a participant in inflammatory and autoimmune reactions.[10] ST2 is defined as the IL-33 receptor, as it binds to IL-33.[11,12] ST2 has two main isoforms: transmembrane or cellular (ST2L) and soluble or circulating (sST2) forms.[13,14] In experimental models, the interaction between IL-33 and ST2L provided the cardioprotective effects, reducing myocardial fibrosis, cardiomyocyte hypertrophy, and apoptosis, as well as improving myocardial function. sST2 avidly binds to IL-33, competing with ST2L.[14] The interaction of sSH2 with IL-33 blocks the IL-33/ST2L system and, as a result, eliminates the cardioprotective effects. sST2 is considered a decoy receptor.[11] Ultimately, a high level of sST2 in blood plasma leads to myocardial fibrosis and dysfunction of the ventricles of the heart.[15] The main sources of sST2 are cardiac fibroblasts and cardiomyocytes in response to stress or injury. Recent studies have demonstrated soluble ST2 to be a strong predictor of cardiovascular outcomes in both chronic and acute heart failure.[14] Consistent with the ADHF data, soluble ST2 has been proven to be useful as a prognostic marker in chronic HF.[16]

The weaknesses of the NT-proBNP diagnostic capabilities, namely that its representativeness depends on some comorbid conditions of patients (impaired renal function, anemia, COPD, obesity), encourages scientists to search for a more stable diagnostic marker for patients with a different EF of CHF. Despite the fact that separate studies have already been conducted [7,15,17] to assess the relationship of sST2 with other characteristics of CHF patients with different LVEF, the results obtained are contradictory.

The purpose of this study was to determine the blood concentration of sST2 and evaluate the correlations between sST2 and the clinical, instrumental, and echocardiographic characteristics of patients with CHFrEF and CHFpEF, as well as to evaluate the sST2 informativeness in comparison with NT-proBNP.

Materials and Methods

The study involved 160 patients diagnosed with CHF (86 men and 74 women, mean age of 72.4±8.7 years), included in the regional register of CHF patients in the Voronezh region. All subjects gave informed consent to participate in the study. According to the LVEF value, the patients were divided into two groups: CHFpEF (EF≥50%) was recorded in 69 people, CHFrEF (EF<50%) - in 91 people. The diagnosis of CHF was established according to 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure.[1] The functional class (FC) of CHF was determined according to the NYHA classification (1994), based on the results of the 6MWT. The non-inclusion criteria were the presence of bronchopulmonary diseases(including asthma, COPD), chronic kidney disease(3b stages and higher), diabetes mellitus or taking hypoglycemic drugs, permanent atrial fibrillation, anemia, diseases of musculoskeletal system (coxarthrosis, gonarthrosis, etc., reducing motor activity), obesity (2-3 classes) and other severe somatic pathologies.

Eight weeks after inclusion in the study (patients were examined weekly by the investigator-cardiologist to monitor the stability of the state/course of CHF), participants underwent a standard examination, which included clinical, laboratory and instrumental research methods. For the purpose of an objective assessment of exercise tolerance, a complex of cardiorespiratory analysis was used, designed for functional medical tests, which allows dynamic determination of blood oxygen saturation, heart rate, respiratory rate and the distance in 6MWT (6MWT-D).

The serum levels of NT-proBNP, sST2, and hs-CRP were determined using an automatic analyzer IMMULITE 2000 (Siemens Diagnostics, USA) and quantitative ELISA kits: NT-proBNP - using the Biomedica human NT-proBNP Sandwich ELISA kit (Austria), sST2 - using the Presage ST2 assay kit (Critical Diagnostics, USA), and hs-CRP - using the SRB-IFA-BEST highly sensitive kit (Vector-Best, Russia).

Comprehensive two-dimensional and Doppler echocardiography were performed using an EPIQ5 ultrasound system (Phillips, USA) equipped with S5-1 Pure-Wave Cardiac Transducer. LV end-systolic and end-diastolic volume and left ventricular ejection fraction (LVEF) were calculated by the biplane Simpson method.[18] Early transmitral inflow velocity (E) and early diastolic mitral annular velocity (e') were determined, and an E/e' ratio was acquired. LAVI and TAPSE were also evaluated.

All data was evaluated with STATGRAPHICS Plus 5.1 Median (Me) and interquartile range (IQR; 25th to 75th percentiles) were calculated. Mann–Whitney U test was used for group comparisons. Group comparisons with respect to categorical variables were performed using chi-square tests or, alternatively, Fisher's exact test when expected cell counts were less than 5. Spearman's correlations were used to assess the relationship between NT-proBNP, sST2, hs-CRP levels with echo parameters. A probability value of $P<0.05$ was considered statistically significant.

Results and Discussion

The median of NT-proBNP level for patients with CHFrEF was 1804(608-4908) ng/L, which significantly exceeded its value in patients with CHFpEF - 980(301-2677) ng/L ($P<0.001$). The sST2 level was also higher in patients with CHFrEF and amounted to 37(21-56) ng/ml, while for the group of patients with CHFpEF this indicator was 21(15-35) ng/ml ($P<0.001$). sST2 levels in both groups increased with increasing NYHA FC.

In the HFpEF group, the average levels of LAVI and LVMI were 43(37–53) ml/m^2 and 115(95–143) g/m^2, respectively. In the HFrEF group, the average levels of LAVI and LVMI were 48(40–73) ml/m^2 and 146(127–184) g/m^2, respectively. A higher level of sST2 in patients with HFrEF, in combination with more pronounced hypertrophy and myocardial remodeling as described above, indicates that the induction of sST2 is caused by the mechanical overload of cardiomyocytes. Consequently, an increase in the level of this

biomarker may reflect myocardial stress, the architectonics changes in the heart during the development of CHF and fibrosis.

With regard to the level of hs-CRP, as a biomarker of endogenous inflammatory processes, its value was 3.4(1.2-8.1) mg/L in patients with CHFpEF, while in patients with CHFrEF it was statistically significantly less - 2.9(1.6-5.4) mg/L (P<0.001).

The correlation analysis of the sST2 levels and other variables in the study groups produced the following results: NT-proBNP and hs-CRP levels had a stronger positive association with sST2 levels in CHFrEF patients (r=0.53, P<0.001 and r=0.48, P<0.001, respectively) than in CHFpEF patients (r=0.40, P<0.001 and r=0.29, P<0.001, respectively). This is consistent with the theory of heterogeneity of pathological processes in the myocardium in patients with different EF.[1]

We found a weak correlation between sST2 and NYHA FC in both groups, and the strength of the correlation slightly increased with an increase in the severity of CHF: FC II (r=0.23 [P=0.007] in CHFrEF; r=0.21 [P=0.003] in CHFpEF); FC III (r=0.25 [P=0.004] in CHFrEF; r=0.24 [P<0.001] in CHFpEF); FC IV (r=0.28 [P<0.001] in CHFrEF; r=0.26 [P=0.006] in CHFpEF).

The results obtained (regardless of the EF) indicated a statistically significant direct correlation between the levels of sST2 and LAVI (r=0.45 [P<0.001] in CHFpEF; r=0.37 [P<0.001] in CHFrEF), the dysfunction of which can be a marker of the severity of CHF.[19]

The study did not reveal a relationship between sST2 levels and LV filling pressure, calculated as E/e' (r=0.09 [P=0.06] in CHFpEF; r=0.11 [P=0.07] in CHFrEF) or LVMI (r=0.16 [P<0.043] in CHFpEF; r=0.08 [P=0.6] in CHFrEF).

In the group of patients with CHFpEF, an inverse correlation was observed between the sST2 level and 6MWT-D (r=-0.69, P=0.007) and LVEF (r=-0.26, P<0.001). In the group of patients with CHFrEF, the level of sST2 also showed an inverse correlation with LVEF (r=-0.43, P<0.001) and 6MWT-D (r=- 0.52, P=0.008) (Table 1).

Correlation analysis of the sST2 and NT-proBNP levels with functional, laboratory, instrumental parameters of patients with CHFpEF and CHFrEF revealed the strengths and weaknesses of both biomarkers. The study found a moderate correlation between sST2 levels and LAVI. In turn, LAVI is the most representative echo indicator for detecting CHF in patients with preserved EF, reflecting the prognosis of the disease.

NT-proBNP has been shown to be an effective biomarker not only for diagnosis but also for prognostic evaluation both for patients with CHFrEF and CHFpEF.[10,20-25] The increased NT-proBNP levels in CHFrEF and HFpEF should be taken as a marker for increased risk of adverse outcomes. However, the predominance of NT-proBNP, as well as sST2 values in patients with CHFrEF, suggests a greater severity of the myocardial remodeling in CHFrEF, confirmed by the results of echocardiography. The data obtained in this study are consistent with the data of other authors on the increased production of NT-proBNP and sST2 in mechanically overloaded cardiomyocytes, which reflects myocardial stress, ventricular remodeling and fibrosis.[26]

For 6MWT-D and LVEF, moderate correlations with both biomarkers were also statistically significant. Therefore, the sST2 level, reflecting exercise tolerance and myocardial contractility, can be used as a diagnostic and prognostic marker, like LVEF, especially in situations when it is impossible to perform functional tests and instrumental studies.

Table 1.

Correlations between sST2 and other variables in patients with CHFpEF and CHFrEF

Variables	sST2				NT-proBNP			
	CHFpEF		CHFrEF		CHFpEF		CHFrEF	
	r	*P*	r	*P*	r	*P*	r	*P*
NT-proBNP	0.53	<0.001	0.40	<0.001				
hs-CRP	0.48	<0.001	0.29	<0.001	0.29	0.007	0.19	0.009
NYHA FC II	0.23	0.007	0.21	0.003	0.22	<0.001	0.32	0.005
NYHA FC III	0.25	0.004	0.24	<0.001	0.26	0.005	0.39	<0.001
NYHA FC IV	0.28	<0.001	0.26	0.006	0.29	<0.001	0.43	<0.001
E/e'	0.09	0.06	0.11	0.07	0.22	0.043	0.17	0.020
LAVI	0.45	<0.001	0.37	<0.001	0.32	0.008	0.07	0.564
LVMI	0.16	0.043	0.08	0.6	0.13	<0.001	0.28	<0.001
LVEF	-0.26	<0.001	-0.43	<0.001	-0.17	<0.001	0.28	0.046
TAPSE	-0.62	0.002	-0.45	0.004	-0.36	<0.001	-0.24	<0.001
6MWT D	-0.69	0.007	-0.52	0.008	-0.44	0.003	-0.49	<0.001

Stronger correlations between sST2 and NT-proBNP with hs-CRP in CHFpEF (as compared to CHFrEF) indicate the importance of the contribution of subclinical inflammation to the formation of fibrosis, myocardial remodeling, and, consequently, to the course of CHF. Further accumulation of facts indicating the role of inflammatory processes in the development of CHF may lead to a change in therapeutic approaches in the management of patients with preserved and reduced LVEF.

This study has not revealed a statistically significant correlation between sST2 levels and LV filling pressure. These results are consistent with current research, which also demonstrated the absence of an association between ST2 levels and parameters of LV diastolic function or LV geometry measured by echocardiography.[27]

The weak direct correlation between sST2 and NYHA FC, in contrast to the direct strong correlation between NT-proBNP and sST2, indicates that sST2, which is quite important both for determining the current state of the patient and for predicting the course of the disease, still cannot be used as a single and universal indicator. In this regard, further comprehensive study of the diagnostic and prognostic capabilities of the sST2 and NT-proBNP biomarkers in CHF patients with different EF is required.

In conclusion, the success of using NT-proBNP both in practical medicine (as a reference for the diagnosis of CHF) and in scientific research is limited by many factors that can affect its level. Active studies in this direction have made it possible to identify biomarkers that can deliver additional diagnostic and prognostic information about the patient's condition. Additional information about the likelihood of adverse outcomes can be obtained from the determination of hs-CRP. Thus, its increase (simultaneously with an increase in NT-proBNP) indicates an inflammatory process in the myocardium, which reflects myocardial remodeling. Moreover, it differs from changes in the heart during its hypertrophy and ischemia.

The analysis of sST2 levels allows us to conclude that the prospects are promising for its implementation in the diagnosis and prognosis of CHF since it is a marker of myocardial stress and ventricular remodeling and fibrosis.

Competing Interests

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Neurology

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Influence of Wrist Tapping on Alpha Rhythm Synchronization in Patients with Juvenile Myoclonic Epilepsy

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Abstract

Background: The aim of this study was to assess the effect of wrist tapping (WT), according to the author's method, on the synchronization of alpha activity in healthy adults and patients with juvenile myoclonic epilepsy (JME).

***Methods and Results*:** The study included 71 people of working age, including 51 clinically healthy volunteers (median age - 39[21;56] years) and 20 patients with JME (median age 27[23;35] years). Under the influence of WT in a state of sensory deprivation, statistically significant changes in the alpha peak frequency (APF) (P<0.001) and alpha power (AP) (P<0.05) were found in healthy adults. In JME patients, there were statistically significant changes in the APF (P<0.05) and AP (P<0.05), as well as a statistically significant increase in the the alpha range width (P<0.05) after WT. WT, according to the author's technique, allows reducing the severity of alpha rhythm synchronization and leads to a shift in the peak frequency of the alpha rhythm in the occipital leads towards the alpha-2 sub-frequency range and a decrease in AP in both healthy volunteers and JME patients. At the same time, the alpha range width in the occipital leads is statistically significantly increased in JME patients.

Conclusion: The nature of the change in the alpha rhythm, in comparison with the control, indicates the phenomenon of resonance with the frequency of the WT rhythm. The WT effect testifies to the prospects of the clinical application of WT in JME, since it was previously shown that if the selected external frequencies enter into resonance with the neurons of the antiepileptic system, then an antiepileptic effect could be obtained.(**International Journal of Biomedicine. 2020;10(4):347-351.**)

Key Words*:* epilepsy • electroencephalography • resonance • wrist tapping • alpha rhythm

Abbreviations

AP, alpha power; **APF**, alpha peak frequency; **ARW**, the alpha range width; **IGE**, idiopathic generalized epilepsies; **JME**, juvenile myoclonic epilepsy; **GTCS**, generalized tonic-clonic seizures; **WT**, wrist tapping

Introduction

Resonance (French *resonance*, from Latin *resono* "I respond") is a frequency-selective response of an oscillatory system to a periodic external influence, which manifests itself in a sharp increase in the amplitude of stationary oscillations when the frequency of an external influence coincides with certain values characteristic of a given system. For linear oscillatory systems, the values of resonance frequencies coincide with the frequencies of natural oscillations, and their number corresponds to the number of degrees of freedom. Under the influence of resonance, the oscillatory system turns out to be especially responsive to the action of an external force. With the help of resonance, even very weak periodic oscillations can be distinguished and/or amplified. Resonant phenomena can lead to both destruction and increased stability

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of mechanical systems—hence, epileptic systems. External stimuli can provoke epileptic seizures, but if the selected frequencies resonate with the neurons of the antiepileptic system, an antiepileptic effect can be obtained. Currently, the theory of resonance is used more and more widely in neurorehabilitation, when the impact of external stimuli with a certain frequency, which is in resonance with the neuronal activity of defense systems, can have a clinically significant therapeutic effect.[1,2] On the other hand, epileptogenesis is based on a hypersynchronous state of neuronal activity in the epileptic focus. This phenomenon is called secondary bilateral synchronization, a phenomenon that blurs the line between focal and generalized epileptic seizures. Synchronization refers to the simultaneous onset of identical waves under several electrodes in one hemisphere of the brain. Bilateral synchronization is a situation in which identical waves appear simultaneously over the homologous parts of both hemispheres.[3] The development of video-EEG monitoring of epileptic seizures together with neuroradiological research methods have made it possible to establish that a clear division of all epilepsies into generalized and focal epilepsies is an abstraction rather than a clinical reality.

For example, the following facts are well known: focal seizures are present in the structure of generalized epilepsy;[4] focal EEG changes in generalized epilepsy have been detected in 30–35% of all cases;[5] focal structural abnormalities are present in IGE;[6] the frontal lobes play a leading role in the pathogenesis of such a form of epilepsy as JME.[7] In IGE, where the frontal cortex plays a leading role in the development of generalized activity, the effect of hypersynchronous neuronal activity is a possibility.

Thus, using the theory of resonance and hypersynchronous neuronal activity, we can, by external influence at certain frequencies, desynchronize neurons in the focus of epileptic activity, achieve the phenomenon of resonance with neurons of the antiepileptic system and reduce the risk of developing generalized and secondary generalized seizures.

In recent years, not only drug-based, but also non-drug methods of epilepsy therapy have been actively studied,[8] among which the method of WT is of great interest;[9] this method leads to a decrease in the severity of anxiety both in healthy volunteers and in patients with epilepsy.[10] However, we have not found any works that study the effect of carpal tapping on the severity of alpha rhythm synchronization, either in healthy people or in patients with epilepsy. Such a study would undoubtedly be of scientific and clinical interest.

The aim of this study was to assess the effect of WT, according to the author's method, on the synchronization of alpha activity in healthy adults and patients with JME.

Materials and Methods

The study included 71 people of working age, including 51 clinically healthy volunteers (median age - 39[21;56] years) and 20 patients with JME (median age 27[23;35] years).

All participants underwent EEG while subjects were sitting in a dark room, fully awake but with the eyes closed. The following are the parameters of the alpha rhythm in the occipital leads (O1, O2): the alpha peak frequency (APF, Hz), the alpha range width (ARW, Hz), and the type of peak of the alpha rhythm (monopeak, beepeak, polypeak). In addition, the alpha rhythm power (AP, μV^2) in the occipital leads was analyzed. The above characteristics of the alpha rhythm were recorded before the manual tapping technique and during the first three minutes after its completion. EEG recording was carried out in a state of sensory (visual and sound) deprivation.

The study of the WT was carried out according to the author's modified methodology, "Method of exogenous rhythmic stimulation influence on an individual human rhythm" [RF patent №2606489 of 10.01.2017]. Modification of the method included carrying out the study of the patients' individual rhythms without the use of exogenous rhythmic stimulation. The study was conducted in the morning with the exclusion of external sensory stimuli (loud sound, bright light) and other people, except the doctor and the volunteer, during the tapping procedure. The temperature regime of the environment was maintained within the range of 22-25 ºC. WT was done with eyes closed. The technique involved wrist tapping on the surface of the device (Android smartphone), followed by the registration of the time parameters of this process in the original program based on the modified technique "Method of exogenous rhythmic stimulation influence on an individual human rhythm." During the task performance, the mechanogram where vertical strokes display the contact of the finger with the screen appears on the screen of the device.

Inclusion criteria for the main group:

- Patients with JME
- Signed voluntary informed consent
- Male and female
- The age period: the youth (males 17-21; females 16-20 years); the first period of middle age (males 22-35 years; females 21-35 years); the second period of middle age (males 36-60 years; females 36-55 years)
- Russian-speaking Europeans

Inclusion criteria in the control group:

- Healthy adults
- Signed voluntary informed consent
- Male and female
- The age period: the youth (males 17-21; females 16-20 years); the first period of middle age (males 22-35 years; females 21-35 years); the second period of middle age (males 36-60 years; females 36-55 years)
- Russian-speaking Europeans

Exclusion criteria:

- Children and adolescents
- Refusal to participate in this study
- Participation in other studies
- Acute and chronic neurological, psychiatric and endocrinological disorders at the time of examination
- Alcohol intake (2 or more drinks during the last 2 weeks)
- Use of narcotic drugs at the time of the study and in history

Volunteers did not receive any payment for participating in this study. The researchers did not receive any payment for conducting the study.

Statistical processing was carried out using the STATISTICA Version 10 (StatSoft, USA). The normality of

distribution of continuous variables was tested by Shapiro-Wilk test. Median (Me), interquartile range (IQR; 25th to 75th percentiles) were calculated. The Wilcoxon test was used to compare differences between two dependent groups. A value of $P<0.05$ was considered significant.

Results

Under the influence of WT in a state of sensory deprivation (Table 1), statistically significant changes in the APF ($P<0.001$) and AP ($P<0.05$) were found in healthy adults. The majority of the subjects (70.6%) showed a change in the peak characteristics (Table 2), of which 43% showed its splitting and transformation into polypeak, and 27.4% - a decrease in the number of peaks. Only 29.4% of subjects presented no changes in the peak characteristics.

Table 1.

Characteristics of alpha rhythm before and after WT in healthy adults (n=51)

Before tapping Me [P_{25};P_{75}]	After tapping Me [P_{25};P_{75}]	*P*
APF (Hz)		
10.2 [9.6; 10.8]	10.6 [10; 11.1]	0.0006
AP (μV^2)		
37.7 [20.9; 81.5]	37 [12; 64.6]	0.013
ARW (Hz)		
1.2 [1; 1.5]	1.2 [0.7; 1.8]	0.2

Table 2.

Changes in the characteristics of the alpha-rhythm peak in the occipital leads before and after WT in healthy adults (n=51)

Peak characteristic		Amount (%)
Before tapping	After tapping	
Remained unchanged		*15 (29.4%)*
Monopeak	Monopeak	3 (20.0%)
Doublepeak	Doublepeak	5 (33.3%)
Polypeak	Polypeak	7 (46.7%)
Increasing the number of peaks		*22 (43.1%)*
Monopeak	Doublepeak	9 (40.9%)
Doublepeak	Polypeak	6 (27.3%)
Monopeak	Polypeak	7 (31.8%)
Reducing the number of peaks		*14 (27.4%)*
Polypeak	Monopeak	5 (35.7%)
Polypeak	Doublepeak	8 (57.1%)
Beepeak	Monopeak	1 (7.1%)

In JME patients, there were statistically significant changes in the APF ($P<0.05$) and AP ($P<0.05$), as well as a statistically significant increase in the ARW ($P<0.05$) after WT (Table 3). The majority of the subjects (75%) showed a change in the peak characteristics (Table 4), of which 35% showed splitting and transformation into polypeak, and 40% - a decrease in the number of peaks. Only 25% of subjects presented no changes in the peak characteristics.

Table 3.

Characteristics of alpha rhythm before and after WT in patients with JME (n=20)

Before tapping Me [P_{25};P_{75}]	After tapping Me [P_{25};P_{75}]	*P*
APF (Hz)		
10 [9.65; 10.7]	10.36 [9.8; 11.05]	0.038
AP (μV^2)		
58.8 [32.1; 47.4]	47.1 [20.7; 105.22]	0.018
ARW (Hz)		
0.9 [-1.75; 1.5]	1.4 [1.0; 2.05]	0.02

Table 4.

Changes in the characteristics of the alpha-rhythm peak in the occipital leads before and after WT in patients with JME (n=20).

Peak characteristic		Amount (%)
Before tapping	After tapping	
Remained unchanged		*5 (25%)*
Monopeak	Monopeak	1 (20%)
Doublepeak	Doublepeak	1 (20%)
Polypeak	Polypeak	3 (60%)
Increasing the number of peaks		*7 (35%)*
Monopeak	Doublepeak	3 (42.8%)
Doublepeak	Polypeak	2 (28.6%)
Monopeak	Polypeak	2 (28.6%)
Reducing the number of peaks		*8 (40%)*
Polypeak	Monopeak	1 (12.5%)
Polypeak	Doublepeak	5 (62.5%)
Doublepeak	Monopeak	2 (25%)

Discussion

The main regulator of biorhythms and the life processes they cause is the brain,[11] the biorhythm of which is associated with the individual characteristics of self-regulation mechanisms and the level of plasticity of neurodynamic processes.[12] Considerable attention is paid to the study of the synchronization of various parts of the cerebral cortex in the processes of regulation, primarily in the range of alpha and beta rhythms, and to changes in the functional state of the body.[13,14] Targeted studies of fMRI in patients with epilepsy have shown that the functional resting networks of the brain

fluctuate on a scale from a second to a minute.[15,16] Thus, the concept of dynamic functional connections is an important aspect of the functional activity of the brain at rest, which looks at variations in functional connectivity over a short period of time. Analysis of dynamic functional connections showed that spontaneous transitions between networks of interacting regions of the brain are highly organized into a hierarchy of two types of meta states: one for cognitive systems of a higher order, and the other for sensorimotor systems.[17] Several studies have shown hyperdynamic activity in some specific functional networks at rest in various subtypes of epilepsy.[18] Aberrant dynamics of functional connections at rest was also observed in IGE with GTCS,[19] but many questions remain not fully understood. Previous studies have provided ample evidence to support the hypothesis that IGE, and JME in particular, is multiregional thalamocortical "network" form of epilepsy,[20] rather than generalized epileptic disorder. In addition, stationary connectomic correlations with cognitive impairment have been found in the thalamo-motor and thalamo-frontal system.[21,22] However, it remains largely unclear whether there is a deficit in dynamic interactions between functional resting networks in JME. The processes of synchronization of the bioelectric activity of the brain in various physiological and pathological states of the body in active wakefulness and in relaxation, as well as in JME, have not been sufficiently studied. A comprehensive study of the processes of synchronization of brain biorhythms, both at rest and with external (exogenous) stimuli, would contribute to the understanding of the processes by which the plasticity of nervous processes and the regulatory function of the brain develop.

In conclusion, wrist tapping, according to the author's technique, allows reducing the severity of alpha rhythm synchronization and leads to a shift in the peak frequency of the alpha rhythm in the occipital leads towards the alpha-2 sub-frequency range and a decrease in AP in both healthy volunteers and JME patients. At the same time, the alpha range width in the occipital leads is statistically significantly increased in JME patients. The nature of the change in the alpha rhythm, in comparison with the control, indicates the phenomenon of resonance with the frequency of the WT rhythm. Taking into account excessive synchronization of large neuronal populations leading to a hypersynchronous state in epilepsy, the technique we have developed can be promising as a method of non-drug therapy and/or prevention of the development of generalized and secondary generalized epileptic seizures on stages of the aura or simple focal epileptic seizures. The WT effect testifies to the prospects of the clinical application of WT in JME, since it was previously shown that if the selected external frequencies enter into resonance with the neurons of the antiepileptic system, then an antiepileptic effect could be obtained. However, confirmation of our hypothesis requires further study with the inclusion of a sample of patients suffering from focal and generalized forms of epilepsy.

Competing Interests

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Sleep Disorders

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Advanced Oxidation Protein Products and the *CLOCK* 3111T/C Single Nucleotide Polymorphism in Menopausal Women with Insomnia

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Abstract

Background: The aim of this study was to assess the dependence of the advanced oxidation protein products (AOPPs) levels on the genotype of the *CLOCK* 3111T/C SNP in Caucasian menopausal women with and without insomnia.

Methods and Results: The study involved 105 Caucasian menopausal women volunteers aged between 45 and 60 years. The Pittsburgh Sleep Quality Index, PSQI, Insomnia Severity Index (ISI), and Epworth Sleepiness Scale (ESS) were employed. The study of the *CLOCK* 3111T/C SNP (rs1801260) was performed by PCR. The blood level of AOPPs was detected by IEMA. Based on the results of a clinical-anamnestic examination, the women were divided into two groups: the main (with insomnia) and the control (without insomnia). Given the small number of women carrying the *CC* genotype of the *CLOCK* 3111T/C SNP, the CC carriers and *TC* carriers were combined into one group as the carriers of the minor 3111C allele.

There were no statistically significant differences in the AOPP levels between carriers of different genotypes (TT genotype and TC+CC genotypes) in controls and patients. A comparative analysis of AOPP levels in the women of the main and control groups showed higher AOPP levels in women with insomnia carrying the TT genotype than in the control of the same genotype (P=0.013).

Conclusion: Insomnia in menopausal women is associated with increased protein oxidation only in carriers of the TT genotype of the *CLOCK* 3111T/C SNP. (**International Journal of Biomedicine. 2020;10(4):352-356.**)

Key Words: *CLOCK* 3111T/C SNP • protein oxidation • insomnia • menopause

Abbreviations

AOPPs, advanced oxidation protein products; **IEMA**, immunoenzymometric assay; **LPO**, lipid peroxidation; **OS**, oxidative stress; **ROS**, reactive oxygen species; **SNP**, single nucleotide polymorphism

Introduction

Many physiological and metabolic processes, including the sleep-wake cycle, are controlled by a circadian system. It is known that this system consists of a central pacemaker, the suprachiasmatic nucleus (SCN), and of peripheral oscillators found in almost all cell types in brain and body that resonate with circadian cues originating from the SCN.[1,2] Disruption of the biological clock function accompanied by the separation of connections between local oscillators in different tissues or the central oscillator, leads to neuroendocrine rhythms and behavior malfunction. Some studies have demonstrated age-related changes in circadian rhythms.[3] Moreover, any circadian system changes increase the risk of developing many pathological conditions, including sleep disorders.[4]

To date, it has been shown that the hormone melatonin plays an important role in the circadian mechanism.[5] Along

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with its regulatory role in the sleep-wake cycle, melatonin is one of the antioxidants that has more pronounced antioxidant properties than vitamin E or glutathione.[6] It is possible that changes in melatonin secretion circadian rhythms in patients with sleep disorders can lead to OS, which is a result of an imbalance between the production of free radicals and the activity of the antioxidant system.[7] In 1994, Reimund proposed the Free Radical Flux Theory of Sleep.[8] Reimund hypothesized that ROS accumulate in the brain during the wake state, and the lower metabolic rate of sleep provides the brain's antioxidant system the opportunity to catch up, neutralizing neuronal ROS down to baseline levels in preparation for the next day's cycle. The role of OS during sleep was later demonstrated not only by experimental investigations[9-13] but also in human studies,[14,15] including menopausal women.[16] The consequences of LPO product accumulation are changes in the metabolism of proteins, fat, carbohydrates, nucleic acids, water, and electrolyte metabolism, which can cause severe tissue damage and reduction in the adaptive capacity of the organism.[7] It can lead to the development of different cardiovascular,[17] endocrine,[18] and mental diseases, sarcopenia,[20] as well as cancer[21] and other pathologies.

In our earlier study, we found that melatonin secretion circadian rhythms are associated with the *CLOCK* 3111T/C SNP in Caucasian menopausal women with insomnia. Early morning hours increased the hormone level registered in TT carriers, which allows considering the 3111T allele as risky in the formation of melatonin circadian rhythm disturbances in these patients.[22] Moreover, it has been shown that some LPO and antioxidant system parameters in Caucasian menopausal women with insomnia depend on the *CLOCK* 3111T/C SNP. However, there is no data about the *CLOCK* 3111T/C SNP and oxidation protein relationships in insomnia.

The aim of this study was to assess the dependence of AOPPs levels on the genotype of the *CLOCK* 3111T/C SNP in Caucasian menopausal women with and without insomnia.

Materials and Methods

Subjects

The study was carried out in compliance with Ethical Principles for Medical Research Involving Human Subjects, Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964, and amended by the 64th WMA General Assembly, Fortaleza, Brazil, October 2013. The study was approved by the Ethics Committee of the Scientific Center for Family Health and Human Reproduction Problems. Written informed consent was obtained from each patient.

The study involved 105 Caucasian menopausal women volunteers (the Russian ethnic group) aged between 45 and 60 years, living in Irkutsk city. They were recruited through personal interviews. Once the women had given their written, informed consent to participate in the study, the research program was conducted and included the following methods: clinical-anamnestic, laboratory and statistical.

Inclusion criteria for the perimenopausal group were age of 45–55 years, oligomenorrhea or amenorrhea during 12 months, and ultrasound criteria: (1) endometrial dysfunction (mismatch of structure and thickness corresponding to the first and the second phases of the menstrual cycle); (2) the depletion of ovarian reserve.

Inclusion criteria for postmenopausal group were age of 56–60 years, amenorrhea ≥12 months, follicle-stimulating hormone level >20 iU/ml, index luteinizing hormone/follicle-stimulating hormone<1, and ultrasound criteria: (1) thin non-functional endometrium, endometrial echo thinner than 5 mm; (2) the lack of ovarian reserve.

Exclusion criteria were exacerbation of chronic diseases, hormone replacement therapy, surgical menopause, the presence of chronic sleep disorders in the history before menopause (insomnia, parasomnia, hypersomnia, obstructive sleep apnea syndrome), the use of hypnotic pills in the previous two weeks, and shift work.

Questionnaires

The Pittsburgh Sleep Quality Index,[23] PSQI, Insomnia Severity Index (ISI),[24] and Epworth Sleepiness Scale (ESS) [25] were employed. These questionnaires indicate the presence of insomnia according to the criteria of the International Classification of Diseases (ICD-10) and of the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV).

The PSQI questionnaire consists of 19 validated questions for assessment of sleep quality and efficiency. The global PSQI score is calculated from seven components: subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleeping medications, and daytime dysfunction. Each question within the component is scored on a 4-point Likert scale of 0-3, with 3 indicating worse outcomes, and the mean was calculated for each component score. The total score ranged from PSQI 0 to 21 points. A global PSQI score 5 indicates a "good sleeper" and >5 indicates a "poor sleeper."

The ISI questionnaire consists of 7 items and identifies the severity of sleep continuity disturbance, defined as difficulty initiating sleep and staying asleep, early morning awakenings, and related distress. The answer to each of the questions is scored on a 5-point Likert scale of 0 (none) to 4 (very severe) and a total score ranging from 0 to 28 points. The ISI results 0-7, 8-14, 15-21, 22-28 points were interpreted as the norm, slight sleep disorders, moderate, and severe, respectively.

The ESS consists of 8 items about usual chances of dozing off or falling asleep while engaged in 8 different activities. The answer to each of the questions is scored on a 4-point Likert scale of 0 (not at all likely to fall asleep) to 3 (very likely to fall asleep) and a total score ranging is from 0 to 24 points. The ESS results 0-3, 3-9, 9-16, 16-24 points were interpreted as norm, insomnia, obstructive sleep apnea syndrome, and narcolepsy, respectively.

Blood collection

Venous blood was sampled from the cubital vein into two tubes with EDTA tripotassium salt between 8:00 a.m. and 9:00 a.m. after 12-h overnight fasting. Venous blood from the first tube was used for molecular-genetic examination. The sample from second tube was centrifuged for 10 min at 1.500 g at 4°C. Blood plasma was kept frozen at -40°C for up to one month until AOPP determination.

DNA testing

Genomic DNA samples were isolated from the peripheral blood leukocytes using the AmpliPrime DNA-Sorb-B reagent kit (NEKSTBIO, Russia). The study of the *CLOCK* 3111T/C SNP (rs1801260) was performed by PCR on the DTprime amplifier (DNA-Technology, Russia) using reagent sets for genotyping polymorphic markers (TestGen, Russia).

AOPPs determination

AOPPs blood level (nmol/l) was detected by IEMA using ImmunDiagnostik AG kits (Germany) on a Bio Tek EL×808 analyzer (USA).

Based on the results of a clinical-anamnestic examination, the women were divided into two groups: the main (with insomnia) and the control (without insomnia). Given the small number of women carrying the CC genotype of the *CLOCK* 3111T/C SNP, the CC carriers and TC carriers were combined into one group as the carriers of the minor 3111C allele. The basic characteristics of the groups are demonstrated in Table 1.

Statistical processing was carried out using the STATISTICA Version 10 (StatSoft, USA). The normality of distribution of continuous variables was tested by Shapiro-Wilk test. Mean±standard deviation (SD), median (Me), interquartile range (IQR; 25th to 75th percentiles) were calculated. The Mann-Whitney U-Test was used to compare differences between two independent groups. A value of $P<0.05$ was considered significant.

Table 1.

The basic characteristics of the groups

Characteristics	Control		Insomnia	
	3111T/T (n=16)	3111T/C+ 3111C/C (n=20)	3111T/T (n=33)	3111T/C+ 3111C/C (n=36)
	n(%)			
Perimenopause	7(43.75)	8(40)	15(45.45)	16(44.44)
Postmenopause	9(56.25)	12(60)	18(54.55)	20(55.56)
Mean±SD				
Age, yr	53.75±5.68	53.35±5.66	54.85±4.99	54.75±5.01
BMI, kg/m^2	27.45±4.27	26.41±3.57	28.14±1.32	27.16±2.23
PSQI (points)	2.32±0.41	2.11±0.15	16.27±2.33*	15.74±3.04^
ISI (points)	3.71±1.81	4.56±1.18	24.41±1.11*	22.91±1.17^
ESS (points)	1.63±0.28	1.03±0.14	7.42±1.25*	6.65±1.98^

**-<0.05 between 3111T/T carriers in control and insomnia*
^-<0.05 between 3111T/C+3111C/C carriers in control and insomnia

Results

AOPPs levels in menopausal women with insomnia and in the control group, regardless of the *CLOCK* 3111T/C SNP, are presented in Figure 1. No differences between control and main group were found.

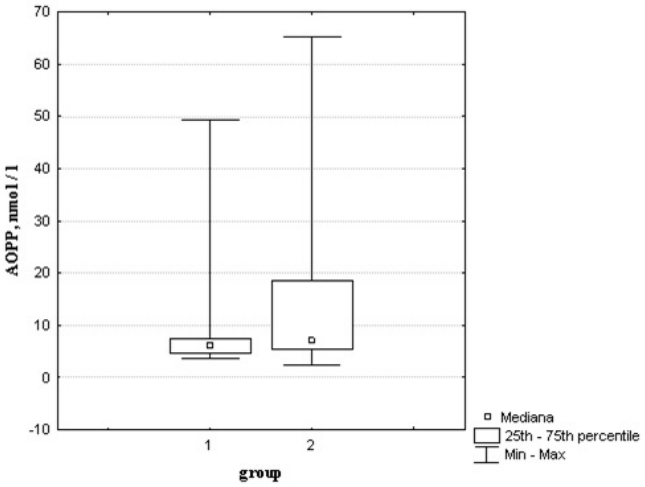

Fig. 1. *AOPP levels in control group and women with insomnia*
Note: 1 - control; 2 – insomnia

AOPPs levels in menopausal women with different genotypes of the *CLOCK* 3111T/C SNP are presented in Figure 2. There were no statistically significant differences in the AOPP levels between carriers of different genotypes (TT genotype and TC+CC genotypes) in controls and patients. A comparative analysis of AOPP levels in the women of the main and control groups showed higher AOPP levels in women with insomnia carrying the TT genotype than in the control of the same genotype (P=0.013).

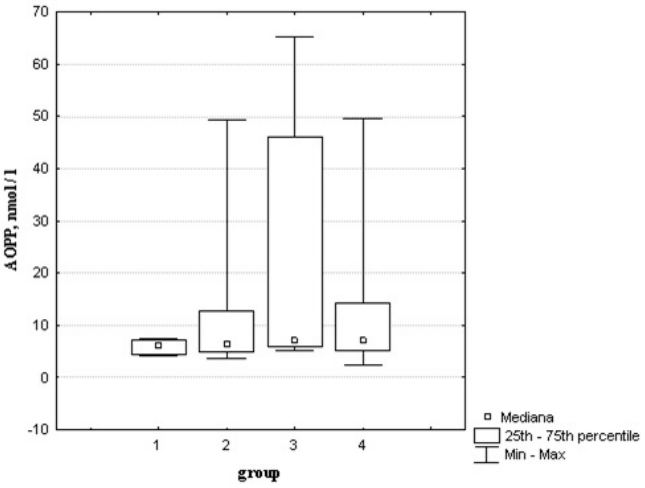

Fig. 2. *AOPPs levels in menopausal women with different genotypes of the CLOCK 3111T/C SNP*
Note: 1 - control, TT-genotype; 2 - control, TC-, CC-genotypes; 3 - insomnia, TT-genotype; 4 - insomnia, TC-, CC-genotypes

Discussion

Proteins are one of the main targets of ROS, and their damage associated with the impossibility of repairing most protein damage exerts a significant effect on cell viability.[26] Damaged proteins can cross-link and be involved in the pathogenesis of various diseases. Intensified oxidative modification of

proteins and increased concentration of AOPPs are confirmed by many experimental investigations in different pathological states, especially those with well-known participation of OS in pathogenesis.[27]

Previously ambiguous results about the relationship between OS and insomnia have been shown in some experimental [12,13] and human studies.[14,15] The results of the studies involving menopausal women with insomnia have demonstrated increased levels of TBARS and similar outcomes with control antioxidant system parameters in patients.[16,28] However, further research showed that higher TBARS levels were identified in menopausal women with insomnia who were TT carriers of the *CLOCK* 3111T/C SNP, while carriers of the minor *C* allele had only primary LPO products at a high level.[29]

The results of our study demonstrated that protein oxidation in menopausal women with insomnia also depends on the *CLOCK* 3111T/C SNP. These data confirm the development of OS in TT carriers with insomnia. A possible reason for this may be a shift in the melatonin secretion rhythms in these patients.[22] In addition, an increase in AOPP levels combined with a decrease in melatonin levels has been demonstrated in several studies.[30] Another possible reason for the development of OS could be differences in activities of proteasome and lysosomal systems for irreversibly damaged protein utilization and amino acid reuse for continuous protein synthesis in the body. It is known that their proteolytic activity significantly decreases with age.[26]

Based on our results, it can be assumed that the 3111C allele is protective for excessive protein oxidation in menopausal women with insomnia. Insomnia in menopausal women is associated with increased protein oxidation only in carriers of the TT genotype of the *CLOCK* 3111T/C SNP. Preventing OS leading to AOPP accumulation and various pathological conditions is important for menopausal women carrying the TT genotype of the *CLOCK* 3111T/C SNP.

Competing Interests

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

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The Results of Bacteriological Examination in Premature Infants with Neonatal Morbidity and Mortality

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Abstract

The purpose of this study was to assess the results of bacteriological studies in children born prematurely and compare the received data with the detected neonatal morbidity.

Methods and Results: Our study included 227 pregnant women at gestational age of 28-36 weeks 6 days, and their newborns. Depending on the gestational age, they were divided into 3 groups. Group 1 included 73 women at gestational age of 28-30 weeks 6 days; Group 2 included 81 women at gestational age of 31-33 weeks 6 days, Group 3 included 73 women at gestational age of 34-36 weeks 6 days. All women underwent an assessment of vaginal microcenosis and the quantitative and qualitative composition of the biotope of the cervical discharge; the newborns underwent bacteriological examination of the auricle, pharynx and anus. Analysis of the results of bacteriological studies shows a significant growth of microorganisms in newborns from mothers of Group 1. The analysis of morbidity among premature infants showed that in each group there were 2 or 3 diseases, mainly of an infectious nature. The main proportion of morbidity (congenital pneumonia and infections of the perinatal period, diseases of the urinary system, neonatal jaundice of premature infants and cerebral ischemia) among newborns was found in Group 1, compared with Groups 2 and 3.

The analysis of the results obtained showed that the low birth weight in preterm labor correlated with the growth of *Staphylococcus epidermidis* in the throat of newborns. Neonatal jaundice of premature newborns was characterized by 100% detection of *Staphylococcus epidermidis* and *Serratia odorifera* in the anus swabs, and *Staphylococcus epidermidis* in swabs from the pharynx and ear. Congenital pneumonia positively correlated with the growth of *Staphylococcus epidermidis*, *E. coli*, *Candida spp*, *Enterococcus faecalis* in the throat swab. The deceased children had a co-infection.

***Conclusion*:** Our study identified the main microorganisms affecting both perinatal morbidity and neonatal mortality: *Staphylococcus epidermidis*, *Enterococcus faecalis*, *E. coli, Candida spp*. It is necessary to note the frequent identification of E. coli strains resistant to the main antibacterial drugs. (**International Journal of Biomedicine. 2020;10(4):357-361.**)

Key Words: premature infants • neonatal morbidity • microbiota • congenital pneumonia

Introduction

The high contamination of the genital tract of pregnant women with conditionally and absolutely pathogenic bacterial and viral microbiota is a factor of high risk for premature birth, and the shorter the gestation period, the higher the frequency of vaginal biotope disorders. The risk of early preterm birth correlates with the presence of infection in the mother. It is the data of the first bacteriological culture that seem important, since after a course of antibacterial therapy, the subsequent results of bacteriological cultures are not very informative for an adequate assessment of the vaginal microbiota.[(1)]

According to the American Association of Obstetricians and Gynecologists, the incidence of membranes rupturing before 37 weeks' gestation ranges from 5% to 35% and complicates 2%-4% of singleton pregnancies.[(2,3)] S. Mamedova, studing the nature and degree of contamination

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of amniotic fluid, has found premature rupture of membranes due to monoinfection, dual infection, and triple infection in 24.4%, 63.3%, and 12.2% women, respectively.[4]

The purpose of this study was to assess the results of bacteriological studies in children born prematurely and compare the received data with the detected neonatal morbidity.

Materials and Methods

This research was carried out in 2017–2018. Our study included 227 pregnant women at gestational age of 28-36 weeks 6 days, and their newborns. Depending on the gestational age, they were divided into 3 groups. Group 1 included 73 women at gestational age of 28-30 weeks 6 days; Group 2 included 81 women at gestational age of 31-33 weeks 6 days, Group 3 included 73 women at gestational age of 34-36 weeks 6 days.

All women underwent an assessment of vaginal microcenosis and the quantitative and qualitative composition of the biotope of the cervical discharge; the newborns underwent bacteriological examination of the ear, pharynx, and anus.

To analyze the nature of the quantitative and qualitative composition of the biotope of the cervical discharge, a tampon-probe and test tubes with a transport medium ("Medical Wire & Equipment," England) were used. Seeding was performed on a series of nutrient media to isolate and cultivate various groups of microorganisms: 5% blood agar based on Brucella agar with the addition of vitamin growth factors to isolate and cultivate anaerobes, mannitol salt agar to isolate and cultivate gram-negative bacteria, and Saburo medium to isolate and cultivate fungi. Blood agar media were cultivated in a thermostat with a high content of carbon dioxide (5-10%). To cultivate anaerobes we used anaerostats (Becton Dickinson, USA). The isolated microorganisms were identified and their sensitivity to antibacterial drugs was determined using the Witek bacteriological analyzer. The results obtained were recorded in accordance with the NCCLS standards (1999–2000). The number of isolated microorganisms was determined by the density of their growth on the sectors of the agar plate.

Bacteriological study of auricular secret and pharynx

The material obtained and transported to the laboratory was seeded on Petri dishes with 5% blood agar, chocolate agar, and yolk-salt agar, on Endo medium, Saburo medium, and in a tube with glucose broth. Seeding on dense nutrient media was carried out metered (according to Gould), which made it possible to quantify the number of grown colonies. Seedings were incubated at 37°C for 24–48 hours, and were examined daily. Plates with 5% blood agar were incubated under conditions with a high CO_2 content. With the appearance of growth on nutrient media, we counted colonies of various morphologies, taking into account their ratio and species identification of microorganisms, as well as determining their sensitivity to antibacterial drugs. A negative result of the study was issued in the absence of growth on all nutrient media for 72–96 hours.

The study was conducted in accordance with ethical principles of the WMA Declaration of Helsinki (1964, ed. 2013) and approved by the RUDN University Ethics Committee. Written informed consent was obtained from all participants.

Statistical analysis was performed using the Statistica 8.0 software package (StatSoft Inc, USA). Baseline characteristics were summarized as frequencies and percentages. Group comparisons with respect to categorical variables are performed using chi-square test. The Spearman's rank correlation coefficient (r_s) was used to determine the strength and direction of the relationship between two variables. The odds ratio (OR), its standard error and 95% confidence interval (CI) were calculated. Logistic regression was used to model dichotomous outcome variables. A value of $P<0.05$ was considered statistically significant.

Results

Analysis of the results of bacteriological studies shows a significant growth of microorganisms in newborns from mothers of Group 1 (Table 1). In general, there were statistically significant differences in the detection of microbial growth between groups, with statistically significant differences between Groups 1 and 2 ($P=0.0068$), Groups 1 and 3 ($P<0.001$), and Groups 2 and 3 ($P=0.0257$).

Table 1.
The results of bacteriological studies in newborns

Detection of microbial growth	Group 1 (n=73)	Group 2 (n=81)	Group 3 (n=73)
Throat swab	48 (65.8%)	41 (50.6%) $P_{1-2}=0.0576$	33 (45.2%) $P_{1-3}=0.0125$
Anus swab	45 (61.6%)	46 (56.8%)	25 (34.3%) $P_{1-3}=0.0009$ $P_{2-3}=0.0051$
Ear swab	42 (57.5%)	40 (49.4%)	30 (41.1%) $P_{1-3}=0.0470$
Growth of different microbial strains in low titers (throat, anus, ear)	63 (86.3%)	58 (71.6%) $P_{1-2}=0.0264$	43 (58.9%) $P_{1-3}=0.0002$ $P_{2-3}=0.0976$

The analysis of morbidity among premature infants showed that in each group there were 2 or 3 diseases, mainly of an infectious nature: congenital pneumonia and infections of the perinatal period, diseases of the urinary system, neonatal jaundice of premature infants and cerebral ischemia. The main proportion of morbidity among newborns was found in Group 1, compared with Groups 2 and 3 (Table 2).

According to the results of bacteriological examination in women, mainly mono- and dual microorganisms were found, less often a combination of 3 and even less often 4 microorganisms.

The analysis of the results obtained showed that the low birth weight in preterm labor correlated with the growth

of *Staphylococcus epidermidis* in the throat of newborns (r_s=0.411, *P*<0.001). Neonatal jaundice of premature newborns was characterized by 100% detection of *Staphylococcus epidermidis* and *Serratia odorifera* in the anus swabs, and *Staphylococcus epidermidis* in swabs from the pharynx and ear (Table 3).

Congenital pneumonia positively correlated with the growth of *Staphylococcus epidermidis* (r_s=0.722, *P*<0.001), *E. coli*(r_s=0.416, *P*<0.001), *Candida spp.* (r_s=0.334, *P*<0.001), *Enterococcus faecalis* in the throat swab. A direct correlation was found between neonatal morbidity and the growth of *E. coli* in the throat of newborns (r_s=0.703, *P*<0.001) and *Serratia odorifera* and *Candida spp.* (r_s=0.314, *P*<0.05) in the anus swabs.

The deceased children had a co-infection (Table 4, 5). For example, in a postnatally dead child from Group 1, we found a moderate growth of *Staphylococcus aureus* in the throat swab, an abundant growth of *Staphylococcus haemolyticu*s in the anus swab and an abundant growth of *Staphylococcus epidermidis* in the ear swab. In Group 2, in 2 dead children, the growth of *E. coli*, resistant to antibiotic therapy, was determined in the throat swab.

Using binary logistic regression analysis, a reliable model (*P*<0.001) was obtained, and based on this the most significant bacteria were identified (Table 6), the detection of which allows predicting an unfavorable pregnancy outcome and the presence of neonatal morbidity.

Table 2.

The main diseases and congenital malformations of children identified in the maternity hospital

Neonatal morbidity	Group 1 (n=73)	Group 2 (n=81)	Group 3 (n=73)
Congenital pneumonia	35 (47.9%)	17 (21.0%) P_{1-2}=0.0004	9 (12.3%) P_{1-3}=0.0000
Other infections of the perinatal period	21 (28.8%)	11 (13.6%) P_{1-2}=0.0204	6 (8.2%) P_{1-3}=0.0014
Low birth weight	9 (12.3%)	22 (27.2%) P_{1-2}=0.0219	34(46.6%)* P_{1-3}=0.0000 P_{2-3}=0.0124
Intraventricular hemorrhage	14 (19.2%)	9 (11.1%)	1 (1.4%) P_{1-3}=0.0004 P_{2-3}=0.0143
Cerebral ischemia	63 (86.3%)	43 (53.1%) P_{1-2}=0.0000	31 (42.5%) P_{1-3}=0.0000
Diseases of the urinary system	17 (23.3%)	6 (7.4%) P_{1-2}=0.0058	3 (4.1%) P_{1-3}=0.0008
Neonatal jaundice of premature infants	26 (35.6%)	42 (66.7%) P_{1-2}=0.0428	32 (43.8%) P_{2-3}=0.3202
Congenital atresia of the esophagus	4 (5.5%)	1 (1.2%)	1 (1.4%)
Hemorrhagic disease	3 (4.1%)	1 (1.2%)	-
Total	73 (100.0%)	54 (66.7%) P_{1-2}=0.0000	37 (50.7%) P_{1-3}=0.0000 P_{2-3}=0.0440

Table 3.

Neonatal morbidity and identified microorganisms in premature newborns

Neonatal morbidity	Throat swab	Anus swab	Ear swab
Congenital pneumonia (n=52)	*Staph. epidermidis* - 42.9%; *Proteus mirabilis* -17.3% *E. coli* - 32.7%; *Enterococcus faecalis* - 28.6% *Candida spp.*- 30.8%; *Staph. warneri* - 26.9%	*Staph. saprophyticus* - 25%	
Other infections of the perinatal period (n=38)	*Staph.epidermidis* -10.5% *Staph.saprophyticus* - 34.2% *Str.viridans* -10.5%	*Serratia odorifera* - 26.3% *Staph. epidermidis* - 31.6%	*Enterococcus faecalis* -13.2%
Low birth weight (n=65)	*Staph. epidermidis* -76.9% *Enterococcus faecalis* - 49.2%	*Staph. saprophyticus* - 26.2%	
Intraventricular hemorrhage (n=25)	*Staph. epidermidis* -24%; *Proteus mirabilis* - 24% *Enterococcus faecalis* - 25%; *Candida spp.*- 50% *Str.viridans* -20%		*Staph. epidermidis* -25%
Cerebral ischemia (n=137)	*Str.viridans* - 28.5%; *Staph. warneri* - 14.3% *Staph. aureus* - 8.8%; *Staph. epidermidis* - 27% *Proteus mirabilis* - 13.9%	*E. coli* - 25.5% *Staph. epidermidis* - 28.6% *Staph. haemolyticus* - 14.3% *Serratia odorifera* - 21.4% *Corynebacterium spp.* - 7.1%	*E. coli* -14.3% *Enterococcus faecalis* -21.4% *Staph. epidermidis* -42.9%
Diseases of the urinary system (n=26)	*Staph. epidermidis* -46.2%; *Staph. warneri* -34.5% *Enterococcus faecalis* -15.4%	*E.coli* - 23.1%; *Staph. epidermidis* -26.9% *Serratia odorifera* - 34.6%	*Enterococcus faecalis* -26.9% *Staph. epidermidis* - 23.1%
Congenital atresia of the esophagus (n=6)			*Serratia marcescens* -50%
Neonatal jaundice of premature infants (n=100)	*Staph. epidermidis* - 50%; *Staph. saprophyticus* -25% *Enterococcus faecalis* -20%	*Staphylococcus epidermidis* - 25% *Serratia odorifera* - 30%	*Staph. epidermidis* – 30%
Hemorrhagic disease (n=4)	*Serratia marcescens* -25%; *Proteus mirabilis* - 50%		

Table 4.

Frequency of bacterial growth and neonatal mortality

	Group1 (n=73)	Group 2 (n=81)	Group 3 (n=73)
Neonatal mortality	8 (11.0%)	6 (7.4%)	-
Throat swab	7 (87.5%)	4 (66.7%)	-
Anus swab	4 (50%)	2 (33.3%)	-
Ear swab	3 (37,5%)	1 (16.7%)	-
Detection of microbial growth	8 (100%)	5 (83.3%)	-

Using the binary logistic regression method, the probability of developing diseases in children was determined, based on the data, by the formula:

$$p = \frac{1}{1 + e^{-z}}$$ where: *e* is the base of the natural logarithm, approximately equal to 2.718

$z = -7.172 + 0.314 \times X1$

In the previous studies,[1,5] we showed the main risk factors leading to premature birth: early sexual debut, inflammatory diseases of the urinary organs, infectious factors, including sexually transmitted infections, reproductive losses in history, anemia, etc. In the present study, the results obtained indicate a significant negative effect of opportunistic flora on perinatal outcomes. Our study identified the main microorganisms affecting both perinatal morbidity and neonatal mortality: *Staphylococcus epidermidis*, *Enterococcus faecalis*, *E. coli*, *Candida spp*.

Table 5.

The results of bacteriological examination in the deceased children

	Group 1 (n=8)	Group 2 (n=6)
Throat swab		
Staph. epidermidis	4 (50%)	2 (33.3%)
E.coli	3 (38%)	2 (33.3%)
Enterococcus faecalis	2 (25%)	1 (16.7%)
Candida spp.	2 (25%)	1 (16.7%)
St.saprophyticus	-	1 (16.7%)
Neisseria spp	-	1 (16.7%)
Staph..aureus	2 (25%)	1 (16.7%)
Proteus mirabilis	1 (13%)	-
Ear swab		
Staph. epidermidis	2 (25%)	2 (33.3%)
E.coli	1 (12.5%)	1 (16.7%)
Enterococcus faecalis	1 (38%)	1 (16.7%)
Staph..aureus	2 (25%)	1 (16.7%)
Proteus mirabilis	1 (12.5%)	-
Anus swab		
Staph. haemolyticus	1 (12.5%)	-
E.coli	1 (12.5%)	1 (16.7%)
Enteroccocus faecalis		1 (16.7%)
Serratia marcescens		1 (16.7%)
Proteus mirabilis	1 (12.5%)	-

Table 6.

Mathematical model for the main microorganisms affecting perinatal morbidity

Statistics	Const.B0	Staph. epirmidis	Enteroccocus faecalis	E.coli	Candida spp
Estimate	-2.507	0.412319	0.349	0.4228	0.034
Standard Error	1.5042	0.167	0.167	0.1367	0.012
t(129)	-0.00783	2.46511	1.995028	3.091231	2.86897
P-value	0.9938	0.0150	0.0496	0.0024	0.0048
-95%CL	-2.98795	0.081387	0.00251	0.152412	0.05832
+95%CL	2.964407	0.74325	0.701628	0.693305	0.21071
Wald Chi-square statistics	6.12E-05	6.076768	3.861334	9.555711	8.230992
P-value	0.993756	0.013702	0.04942	0.001995	0.004121
Odds ratio (unit ch)	0.988297	1.510316	1.418444	1.526318	1.366072
-95%CL	0.050391	1.084791	1.07496	1.164639	1.043348
+95%CL	19.38321	2.102759	2.017034	2.000315	1.989344

It is necessary to note the frequent identification of *E. coli* strains resistant to the main antibacterial drugs. These results will help to predict the risk of premature birth in a timely manner, and to carry out adequate antibiotic therapy, thus increasing the possibility of a favorable outcome of premature birth and prolongation of pregnancy with timely successful treatment.

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Competing Interests

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

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Features of the Immune System Functioning with Persistence of Infectious Agents in Women with Chronic Endometrial Inflammation and Reproductive Disorders

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Abstract

The objective of our study was to investigate the specific features of immune status indicators in women of reproductive age with chronic endometritis (CE) and reproductive disorders.

Methods and Results: The observational study involved 81 women of reproductive age with reproductive disorders. The main group (MG) included 50 women with CE (mean age of 29.2±5.34 years). The control group (CG) consisted of 31 fertile women also of reproductive age(mean age of 30.7±5.9 years). MG was divided into the following subgroups: Subgroup A (SubA) included 31 patients with verified CE and an isolated infectious agent from endometrial tissue: Subgroup B (SubB) included 19 patients with verified CE and the absence of an infectious agent in the endometrial tissue. Endometrial aspiration pipe biopsy was performed on days 4-9 of the menstrual cycle (middle proliferative phase) using a disposable intrauterine probe (Taizhou Kechuang Medical Apparatus Co., Ltd, China) followed by histological examination of endometrial tissue. Laboratory diagnostics for sexually transmitted infections (STIs) was performed using the bacterial culture method. For the diagnosis of viral infection (HPV, HSV, CMV), cervical samples were studied using PCR. If STIs were detected, the patients were excluded from further research. Ultrasound examination of the pelvic organs was performed using the Aloka-5500 device with a 7MHz vaginal probe in two-dimensional visualization mode. The concentration of cytokines (IL-1β, INF-γ, TNF-α, ILs-4,6,8,10) in the endometrium was determined using the Protein Contour test systems (Russia) and Multiskan EX ELISA Analyzer (Germany). The percentages and absolute counts of blood lymphocytes (CD3+, CD3+/CD8+/CD45+, CD19+/CD45+, and CD16+/CD56+/CD45+ cells) were determined by the method of indirect immunofluorescence with monoclonal antibodies using the BD FACSCalibur flow cytometer (USA).

We found a significant increase in the blood concentrations of CD3+ cells, CD3+/CD8+/CD45+ cells, and CD19+/CD45+ cells and a decrease in the levels of CD16+/CD56+/CD45+ cells, microbicidal activity of oxygen-dependent function of neutrophils, and phagocytic activity of neutrophils, as well as a significant decrease in the levels of IgA, IgM, and IRI in MG, compared to CG. In SubA, compared to SubB, we found a significant decrease in CD3+ cells and CD19+/C45+ cells and a slight increase in immunoregulatory index. The concentrations of tissue cytokines in women of MG were characterized by a 3-fold increase in the level of pro- and anti-inflammatory cytokines (IL-1β, ILs - 4, 6, 10, and INF-γ), and a 4-fold increase in the levels of TNF-α and IL-8, compared to CG. In SubA, in comparison with SubB, a significant decrease in anti-inflammatory cytokines (ILs-4,10) and chemokine IL-8 was revealed against the background of a significant increase in the concentrations of INF–γ.

Conclusion: The results obtained indicate changes in the reactivity of the immune system in women with reproductive disorders and chronic inflammation in the endometrium. The most pronounced changes in the local immunity indicators are observed when opportunistic pathogens are detected in the endometrial tissue.(**International Journal of Biomedicine. 2020;10(4):362-368.**)

Key Words: chronic endometritis • immunity • reproductive disorders

Abbreviations

CE, chronic endometritis; **CIC**, circulating immune complexes; **NBT-test**, nitroblue tetrazolium test; **HSV**, herpes simplex virus, **IL**, interleukin; **IRI**, immunoregulatory index; **OPs**, opportunistic pathogens; **STIs**, sexually transmitted infections; **PCR**, polymerase chain reaction; **NK**, natural killer.

Introduction

Chronic endometritis (CE) is a poorly understood pathology. The high risk of reproductive dysfunctions, and complicated pregnancy and childbirth, in CE determines the need for a comprehensive study of this pathology. CE is mainly found in women of reproductive age, where its frequency ranges from 3% to 73%.[1-3] Patients with a chronic, oligosymptomatic inflammatory process in the small pelvis are particularly challenging for diagnosis and treatment. Evaluation of clinical manifestations revealed the absence of any clinical symptoms of CE in 35%–40% of patients.[4] It should be noted that the information content of using the minimum criteria for the diagnosis of pelvic inflammatory disease, which were proposed by the CDC (USA, 1993), in cases of CE is observed only in 33%.[4,5]

Traditional treatment regimens have not been very effective; according to some authors, their effectiveness does not exceed 58%-67%.[6-9] Prescribing antibiotics to patients with sluggish forms of CE is equated with drug aggression. In the absence of a proven persistence of a causally significant infectious agent, starting treatment with antimicrobial drugs is not recommended, since histologically proven chronic endometritis is based on the autoimmune nature of inflammation.[10] A number of studies have shown that the use of routine diagnostic methods does not allow identifying an etiological factor in 40%-70% of cases of CE.[11,12]

Infection is the basis of CE. Cicinelli et al.[13] analyzed 438 cases of hysteroscopically diagnosed CE, and reported that 73.1% exhibited ≥1 positive pathogen finding. Most commonly, CE is provoked by Enterococcus faecalis, Enterobacteriaceae, Streptococcus spp., Staphylococcus spp., Gardnerella vaginalis, and Mycoplasma spp., as well as genital pathogens associated with STIs (Ur. urealyticum, Chl. trachomatis, and Neisseria gonorrhoeae). Interactions between infectious agents and the endometrial environment are a major concern in the treatment of infertility, miscarriage, and preterm labor.[14] The main issue of CE requiring study is the interaction between microorganisms and endometrial immunity rather than just the presence of microorganisms in the endometrium.

In a study performed by Matteo et al., the secretory endometrium of infertile women with CE displayed a significantly lower percentage of CD56+CD16- and of CD56(bright) CD16- cells (47.8% vs. 30.1% and 79.5% vs. 67.3%, respectively; $P<0.01$) than a group of patients without CE, while the percentage of CD3+ cells was significantly higher (25% vs. 10.5%; $P<0.01$).[15] Kitaya and Yasuo [16] reported that lymphocyte B cell levels were elevated in the endometrium of patients with CE, and they also observed the abnormal expression of paracrine mediators, such as adhesion molecules and chemokines. Tortorell et al. found that IL-6, IL-1β, and TNF-α levels were markedly higher in menstrual effluents of infertile women with CE than in those of control subjects.[17]

Complex interactions between the endocrine and immune systems govern the key endometrial events of implantation and menstruation. In contrast to other tissue sites, cyclical endometrial inflammation is physiological. However, dysregulation of this inflammatory response in the presence of opportunistic pathogens can lead to endometrial disorders.[4,6,18-22]

The objective of our study was to investigate the specific features of immune status indicators in women of reproductive age with CE and reproductive disorders.

Materials and Methods

The observational study involved 81 women of reproductive age with reproductive disorders. The main group (MG) included 50 women with CE (mean age of 29.2±5.34 years). The control group (CG) consisted of 31 fertile women also of reproductive age(mean age of 30.7±5.9 years).

MG was divided into the following subgroups:

- Subgroup A (SubA) included 31 patients with verified CE and an isolated infectious agent from endometrial tissue.
- Subgroup B (SubB) included 19 patients with verified CE and the absence of an infectious agent in the endometrial tissue.

The criteria for inclusion in MG were the absence of pregnancy in regular sex life without contraception for a year or more or miscarriage during the last year, the diagnosis of CE verified by a histopathological examination. Exclusion criteria were the presence of causes for reproductive disorders: endocrine, genetic, hemostasiological, and immunological disorders, including male infertility.

The patients were examined according to the standards of infertility examination, including questionnaires, as well as general clinical, gynecological, and laboratory instrumental examinations. Endometrial aspiration pipe biopsy was performed on days 4-9 of the menstrual cycle (middle proliferative phase) using a disposable intrauterine probe (Taizhou Kechuang Medical Apparatus Co., Ltd, China) followed by histological examination of endometrial tissue. Laboratory diagnostics for STIs (N. gonorrhoeae, T. vaginalis, Ur. urealyticum, M. hominis, M. Genitalium. Chl. trachomatis) was performed using the bacterial culture method. For the diagnosis of viral infection (HPV, HSV, CMV), cervical samples were studied using PCR. If STIs were detected, the patients were excluded from further research. Microbiological studies of the vaginal biotope were carried out in accordance with the guidelines for research methods used in clinical and diagnostic laboratories of medical and preventive institutions. Ultrasound examination of the pelvic organs was performed using the Aloka-5500 device with a 7MHz vaginal probe in two-dimensional visualization mode. The concentration of cytokines (IL-1β, INF-γ, TNF-α, ILs-4,6,8,10) in the endometrium was determined using the Protein Contour test systems (Russia) and Multiskan EX ELISA Analyzer (Germany).

The percentages and absolute counts of blood lymphocytes (CD3+, CD3+/CD8+/CD45+, CD19+/CD45+, and CD16+/CD56+/CD45+ cells) were determined by the method of indirect immunofluorescence with monoclonal antibodies using the BD FACSCalibur flow cytometer (USA).

The study was carried out in compliance with Ethical Principles for Medical Research Involving Human Subjects, Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964, and amended by the 64th WMA General Assembly, Fortaleza, Brazil, October 2013. The study was

approved by the Ethics Committee of the Scientific Center for Family Health and Human Reproduction Problems. Written informed consent was obtained from each patient.

Statistical processing was carried out using the STATISTICA Version 10 (StatSoft, USA). The normality of distribution of continuous variables was tested by Shapiro-Wilk test. The mean (M) and standard error of the mean (SEM) were calculated. The Mann-Whitney U-Test was used to compare differences between two independent groups. Group comparisons with respect to categorical variables are performed using chi-square test. Spearman's rank correlation coefficient was calculated to measure the strength and direction of the relationship between two variables. A value of $P<0.05$ was considered significant.

Results

In the medical history of the MG women, compared to CG, there were significantly higher cases of trichomoniasis ($P=0.000$), Chlamydia ($P=0.000$), ureaplasmosis ($P=0.001$), HSV ($P=0.014$), and candidiasis ($P=0.03$). In the structure of concomitant pathology, ENT (Ear, Nose, and Throat) disorders ($P=0.04$), kidney diseases ($P=0.002$), gastrointestinal disease ($P=0.002$), and allergic diseases ($P=0.001$) were diagnosed more often in MG.

In MG, a high frequency of opportunistic pathogens (OPs) was observed both in the cervical canal and in the endometrium. The detection rate of OPs in the cervical canal was 42%. Monoinfection was found in 8% of cases; a mixed infection with a predominance of an association of three types of microorganisms was found in 34% of cases. The number of microorganisms was in the range of 10^4–10^6 CFU/ml. The following OPs were isolated from the cervical canal: Ur. urealyticum (22%), coagulase-negative staphylococci (mainly S. epidermidis - 18%) with pronounced pathogenic properties in the form of hemolytic activity, and fungi of the genus Candida (18%0, Proteus (16%), and E.coli (10%). Among viral infections, CMV, HSV, and high-risk HPV were identified in 8% for each pathogen. In CG, Ur. urealyticum, Candida, E. coli, M. hominis, Streptococcus, Citrobacter spp., Enterococcus faecalis, and low-risk HPV were detected as monoinfection in isolated cases.

The detection rate of microflora in the endometrium was 60%: monoinfection in 12% of cases and the presence two or three types of microorganisms in 48%. The number of microorganisms was in the range of 10^4–10^6CFU/ml. The species diversity of the endometrial microflora was represented by the groups of enterococci (*E.coli* - 28%, *Enterococcus faecalis* - 16%, *Bacteroides spp.* - 8%), staphylococci (*Staphylococcus epidermidis* - 18%, *Staphylococcus haemolyticus* - 8%, *Staphylococcus* aureus - 8%) and Streptococcus in 4% of cases, and by the absence of these pathogens in the control group.

Table 1.
The parameters of the cellular and humoral immune response in patients of study groups

Immunity indicators	MG (n=50)	SubA (n=31)	SubB (n=19)	CG (n=31)
	1	1/a	1/b	2
CD3+/CD45+	72.56±7.5	72.53±8.7	72.33±7.6	71.09±4.6
CD3+	1693±338.4*	2254.52±373.5^	2574.73±202.9	1462±348.4
CD3+CD8+/CD45+	28.6±4.8*	27.64±4.5	28.44±4.7	22.25±3.4
CD3+CD4+/CD45+	43.1±5.4	45.11±0.2	43.29±5.6	45.93±5.3
CD3+CD4+CD8+/CD45+	0.80±0.11	0.64±0.7	0.81± 0.8	0.74±0.17
CD16+56+/CD45+	12±4.7*	12.17±5.2	12.14±4.7	13.6±4.7
CD19+/CD45+	18±5.9*	11.88±2.1^	15.14±2.6	12.13±3.4
Phagocytosis	52.6±10.4*	52.70±11.4	52.60±10.6	61.45±8.3
NBT-test spon.	5.4±3.2*	5.35±3.5	5.31±3.2	9.29±7.7
NBT-test ind.	28.±11.2	29.29±10.2	28.87±11.4	32.35±11.8
IgG, g/l	13.4±5.7	13.73±6.0	13.36±5.8	14.26±4.2
IgA, g/l	1.46±0.8*	1.47±0.8	1.48±0.8	2.3±1.1
IgM, g/l	2.0±0.9*	1.90±0.9	2.00±0.9	2.6±0.8
CIC	37.0±11.1	36.11±11.1	37.10±1.3	40.74±14.1
IRI (CD4+/CD8+)	1.5±0.5*	1.68±0.7	1.55±0.6	2.12±0.4

$*P_{1-2}<0.05$, $^P_{1/a-1/b}<0.05$

Table 2.
The levels of cytokines in the endometrial tissue in patients of study groups

Indicators of local immunity	MG (n=50)	SubA (n=31)	SubB (n=19)	CG (n=31)
	1	1/a	1/b	2
IL-1β, pg/ml	64.9±6.25*	64. 97±39.25	62.85±43.54	23.64±3.37
IL-4, pg/ml	38±15.5*	22.38±20.12^	38.22±13.71	13.71±1.93
IL-6, pg/ml	87.1±9.98*	85.32±39.91	87.00±71.15	39.53±3.81
IL-8, pg/ml	110.6±10.3*	92.82±48.24^	112.98±12.80	23±2.42
IL-10, pg/ml	53.1±7.0*	38.67±39.46^	51.34±51.35	26.67±4.61
INF-γ, pg/ml	53.82±4.38*	100.65±76.29^	73.46±65.33	25.75±4.24
TNF-α, pg/ml	48.5±7.27*	58.00±54.25	48.17±53.34	9.48±0.85

$*P_{1-2}<0.05$, $^P_{1/a-1/b}<0.05$

The immunity parameters in patients of both study groups are presented in Tables 1-2. We found a significant increase in the blood concentrations of CD3+ (T cells), CD3+/CD8+/CD45+ (total T cells and suppressor/cytotoxic T-lymphocytes), and CD19+/CD45+ B cells and a decrease in the levels of CD16+/CD56+/CD45+ (NK cells), microbicidal activity of oxygen-dependent function of neutrophils, and phagocytic activity of neutrophils, as well as a significant decrease in the levels of IgA, IgM, and IRI in MG, compared to CG. In SubA, compared to SubB, we found a significant decrease in CD3+ T-lymphocytes and CD19+/C45+ B-lymphocytes and a slight increase in IRI.

The levels of cytokines produced by immunocompetent cells were determined in the endometrial tissue (Table 2). The concentrations of tissue cytokines in women of MG were characterized by a 3-fold increase in the level of pro- and anti-inflammatory cytokines (IL-1β, ILs - 4, 6, 10, and INF-γ), and a 4-fold increase in the levels of TNF-α and IL-8, compared to CG.

In SubA, in comparison with SubB, a significant decrease in anti-inflammatory cytokines (ILs-4,10) and chemokine IL-8 was revealed against the background of a significant increase in the concentrations of INF–γ.

Correlation analysis revealed an inverse correlation between T lymphocytes and B lymphocytes (r=-0.40) in MG, compared to a direct correlation in CG (r=0.61). In MG, we found the appearance of new correlations between NK cells and NBT-test spontaneous(r=-0.51), and between phagocytosis and NBT-test induced (r=0.67), as well as weak negative correlations between IL-1 and CD3+ lymphocytes (r=- 0.28) and NBT-test spontaneous (r=-0.32). Weak negative correlations were also found between INF-γ and NK cells (r=-0.28), IL-4 and CIC (r=-0.39). IL-4 positively correlated with CD19+ lymphocytes (r=0.32). At the same time, a negative correlation was found between IL-10 and CD19+ lymphocytes (r=-0.19) and positive correlations between IL-10 and IgA (r=0.35) and IgM (r=0.34).

Discussion

A definitive diagnosis of CE can only be made histologically and is noted by the presence of multiple plasma cells in the endometrial stromal area.[23-26] In this connection, CE may describe the condition in which immune cells monitor some aberrant pathogens, which reside in the uterine cavity for a long period, and regulate them to prevent the progression to intense inflammation.[27,28] It is possible that CE is a state with old inflammation after acute endometritis.[29,30]

In CE women with infertility, we revealed the activation of cellular immunity (a significant increase in the blood concentrations of CD3+, CD3+CD8+/CD45+, and CD19+/CD45+ cells and a decrease in the levels of CD16+CD56+/CD45+ cells, microbicidal activity of oxygen-dependent function of neutrophils, and phagocytic activity of neutrophils, and IRI), in comparison with CG women.

Regarding humoral immunity, there was a significant decrease in IgA and IgM, which are necessary for maintaining the first line of immune defense of the mucous membranes from viral infection ($P<0.05$).[31] These data correlate with information about the absence of intersystem connections with the parameter of total phagocytosis activity that indicates an imbalance in the protective systems. A decrease in the oxygen-dependent function of neutrophils (NBT-test spontaneous) confirms the chronicity of the inflammatory process.[32]

The role of IgA in the immune defense is largely determined by its interaction with immunocompetent cells that carry out cell-mediated defense reactions(phagocytosis, cytotoxic effects, etc.).[33] It has been shown that there is a direct relationship between the activity of phagocytosis and the resistance of the organism to infection: the more active the phagocytosis in relation to microbes, the more pronounced the immunity to them, and *vice versa*.[34]

It is known that some stages of phagocytosis can be actively suppressed by microbes or be defective as a result of genetic disorders, which in both cases leads to the inability to effectively remove microorganisms and, as a result, chronic inflammation. The results obtained indicate a decrease in the functional activity of the immune system, reflecting the secondary immunodeficiency state, and are more characteristic of a chronic sluggish inflammatory process with reduced antiviral protection from the mucous membranes.

An additional antigenic load in the verified CE with OPs leads to the activation of antigen-specific mechanisms of the immune response with the formation of further autoimmune reactions. An increase in the expression of all studied cytokines (IL-1β, IL-4, 6, 10, INF-γ,TNF-α , IL-8) in CE women, in comparison with CG, indicates the activation of immunocompetent cells in response to the formed chronic inflammation.[34-36] It is believed that a high concentration of IL-1 affects the systemic inflammatory response by inducing the synthesis of serum amyloid P in the liver that subsequently leads to the stimulation of IL-6 and the production of neutrophils in the bone marrow.[37]

Moreover, IL-1 also modulates the secretion of prostaglandins in the endometrium. Endometrial cells respond to the presence of bacteria by producing prostaglandins, in particular prostaglandin E2.[38,39] In addition, IL-1 in the endometrial tissue promotes the formation of endotoxin by bacteria and viruses and increases degranulation processes.

IL-6 is a pleiotropic cytokine that regulates multiple biological processes, including the development of inflammation, immune responses, and the acute phase of chronic process exacerbation.[32] IL-6 also plays an important role in the processes of chronic inflammation, in particular, in infectious processes of bacterial etiology; it also participates in the activation of specific antibody synthesis in the second phase of the immune response.[32]

IL-8 enhances the directed migration of leukocytes to the inflammatory focus and, together with other cytokines, increases their functional activity aimed at eliminating pathogens.[40] At the same time, pro-inflammatory cytokines activate the metabolism of connective tissue and stimulate the proliferation of fibroblasts and epithelial cells necessary for healing and restoring tissue integrity.

An increase in the level of IL-4 affects the activity of NK cells of the endometrium and reflects the activity of the humoral immune response and the synthesis of antibodies by increasing the level of sIgA.[33,40] IL-4 is one of the key inducers

of the development of an autoimmune response; it is involved in the development of proliferative and fibrotic processes.

The results obtained in our study indicate changes in the immune response that are characteristic of inflammation in CE women. It is well known that the power of the immune response is determined by the antigen load.[35,41] In the presence of OPs, we noted the more pronounced changes in the local immune response. The presence of an infectious agent in the endometrium was characterized by multidirectional changes in cytokine levels, which were expressed by a significant increase in the concentration of TNF-α and INF-γ and by a significant decrease in ILs-4,10 and IL-8 (P<0.05). It is known that an increased immune response during the presence of an infectious agent is associated with a higher level of mRNA expression encoding TLR4 and TLR2, which recognize bacterial LPS and lipopeptides, respectively, as mechanisms of bacterial persistence.[42-44]

In the CE endometrium, there was a significant (1.5-fold) increase in the concentration of INF-γ, relative to the data obtained in CG (P<0.05). INF-γ is the most important endogenous immunomodulator necessary for the development of a specific immune response. It is known that in the late stages of acute inflammation and in chronic inflammation, INF–γ enhances the secretion of antibodies, including autoreactive ones.[45,46] Fournier and Philpott [47] showed that several innate immunity receptors may be implicated in host defense against S.aureus. The ability of peptidoglycan and lipoteichoic acid isolated from Staphylococcus aureus to induce the release of TNF-α, IL-6, and IL-10 by T cells and monocytes was determined by Wang et al.[48] In turn, sIgA and INF-γ are associated with the level of TNF-α, which is one of the key cytokines in implementing the antiviral immune response, as well as in regulating the intensity of inflammation and the effectiveness of immune defense.[32,34] There is a study showing the inhibitory effect of TNF-α on the growth of HSV-infected cells, which is enhanced by the action of INF-γ.[49]

A pronounced increase in the chemokine genes, found after E.coli inoculation, can lead to the recruitment of neutrophils, monocytes, and T-lymphocytes.[38,50] We noted a decrease in IL-8 and the microbicidal activity of neutrophils (P<0.05). This indicates that the neutrophil impact is insufficient, the development of an adequate immune response is slowed down, and therefore the infectious agent that contributes to its persistence is not fully suppressed.

Pro-inflammatory reactions, in order to avoid excessive immune activation by bacteria, including the effects of IL-1, TNF-α, and IL-6, depend on anti-inflammatory mediators such as IL-10, transforming factor 1-β, and prostaglandin E2.[46,51,52]

Some researchers have shown that genes for anti-inflammatory cytokines (IL-10 and IL-13) were expressed at very low levels and there was no significant increase in them in the absence of infection, while the expression of these cytokines increased in response to E.coli invasion. This activity of anti-inflammatory cytokines prepares the immune response for rapid suppression of pro-inflammatory cytokines to prevent an excessive inflammatory response.[15,53]

A decrease in ILs-4,10 in response to the activity of an infectious agent in our study indicates the development of an inadequate, pronounced, local inflammatory reaction in the endometrial tissue with a deficiency of anti-inflammatory cytokines, which may be one of the mechanisms of long-term persistence of the infection in the endometrial tissue.

The presence of a persistent infection characterizes a decrease in the organism's resistance to colonization. It determines the outcome of inflammation (infection). Its decrease is caused by factors that lead to changes in the normoflora—the activity of OPs and/or a decrease in the number of lactobacilli. It is possible that the isolated OPs from the endometrium have an anti-lactoferrin activity, which is the mechanism for maintaining their existence and persistence. According to some studies, the maximum percentage of women with "deep dysbiosis," when the density of lactobacilli is insignificant or not registered by cultural methods, was observed in 49% of women with chronic pelvic inflammatory diseases. In 53% of women with infertility and miscarriage, the complete absence of lactobacilli was also observed.[54]

In conclusion, the results obtained indicate changes in the reactivity of the immune system in women with reproductive disorders and chronic inflammation in the endometrium. The most pronounced changes in the local immunity indicators are observed when opportunistic pathogens are detected in the endometrial tissue.

Competing Interests

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Reproductive Health

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Sperm Quality and Oxidative-Antioxidant Profile in Men Living in Different Regions of Siberia

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Abstract

Background: The aim of this study was to assess the sperm quality and oxidative-antioxidant profile in men living in different regions of Siberia.

***Materials and Methods*:** The study involved 125 men of reproductive age: 40 men (mean age of 24.8 years) living in Ulan-Ude, 35 men (mean age of 24.1 years) in Irkutsk, and 50 men (mean age of 24.6 years) in Novosibirsk. All men belonged to the Caucasian race and were physically healthy. Methods of standard clinical examination of fertile and infertile men included: an ultrasonic scan of scrotum and prostate, macroscopic and microscopic examination of ejaculate, and biochemical analysis. The semen analysis was *performed in accordance* with the WHO recommendations (2010). The study of sperm quality included measuring the volume and pH of the ejaculate, the concentration of spermatozoa, the proportion of motile sperm of categories A and B, and lipid peroxidation-antioxidant activity products. Of the participants in the study, the group of somatically healthy men living in Irkutsk had the highest sperm count (mln per ml): 1.5 times more than men in Novosibirsk and 1.3 times more than men in Ulan-Ude. At the same time, in the group of men in Irkutsk, a significant increase in the concentration of α-tocopherol was revealed: 1.6 times more than the men in Ulan-Ude and 1.8 times more than the men in Novosibirsk.

Conclusion: The conducted studies of the quality of ejaculate and the characteristics of LPO processes in men from different cities of Siberia show that place of residence and ecological-geographical position are not the main reasons determining reproductive dysfunction, but can be important factors affecting the functioning of the reproductive system and determining the heterogeneity of male infertility in conditions of anthropogenic pressure. (**International Journal of Biomedicine. 2020;10(4):369-372.**)

Key Words: antioxidant defense • men • spermatogenesis • ethno groups

Abbreviations

AOD, antioxidant defense; **CD,** conjugated dienes; **LPO**, lipid peroxidation; **MDA**, malondialdehyde**; ROS**, reactive oxygen species; **TAA,** total antioxidant activity; **TBARs**, thiobarbituric acid reactants.

Introduction

Human health is formed as a result of the complex interaction between the hereditary and constitutional characteristics of an organism and between the organism and nature and society. Geographic and ethnic variability of the norm and pathology play an important role.[1-3] The results of many years of fundamental and clinical research indicate that differences exist in the most important physiological constants of the body in the functioning of the reactions of the neuro-immune-endocrine system to the impact of inadequate exogenous and endogenous factors, as well as in the morphological characteristics and adaptive shifts in various environmental conditions.[4-8] Male subfertility, deviations from the norm in sperm counts, and infertility are very common phenomena at the present time.[8,9] The works of foreign researchers of the last century, as well

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as contemporaries, indicate that the lack of vitamins and microelements is one of the reasons for the progressive decline in spermatogenesis indicators.[1,8,10] At all times, nutrition and the ability to reproduce offspring have been closely interrelated. It is known that due to changes in the habitat, and to the nature of nutrition and lifestyle in a modern person, there is a lack of vitamins and microelements (or a violation of their metabolism), which is largely responsible for disorders of the reproductive system.[2,4] Another factor affecting the decrease in male fertility is the hyperproduction of ROS.[11-13] ROS damage the sperm membrane, which leads to a decrease in sperm quality.[10,12] At the stationary level, lipid peroxidation processes are maintained due to the work of the AOS, which includes enzymatic and non-enzymatic links. Disruption of antioxidant protection leads to the development of free radical damage to the structure of spermatogenesis cells, including the DNA membrane and intracellular proteins.[14-16] As a result, there is a violation of the structure and functional qualities of spermatozoa, which subsequently leads to their death. The ratio of the activity of oxidative processes and antioxidant protection largely determines the intensity of metabolism, the adaptive capabilities of the organism and the risk of various pathological conditions.[7,18,19] Since the study of regional features that affect the health status of the population living in a certain territory is of practical importance for maintaining the health of the population, the study of adaptive reactions in practically healthy men living in various regions of Siberia is of undoubted relevance.

The aim of this study was to assess the sperm quality and oxidative-antioxidant profile in men living in different regions of Siberia.

Materials and Methods

The study involved 125 men of reproductive age: 40 men (mean age of 24.8 years) living in Ulan-Ude, 35 men (mean age of 24.1 years) in Irkutsk, and 50 men (mean age of 24.6 years) in Novosibirsk. All men belonged to the Caucasian race and were physically healthy. Methods of standard clinical examination of fertile and infertile men included: an ultrasonic scan of scrotum and prostate, macroscopic and microscopic examination of ejaculate, and biochemical analysis. The semen analysis was performed in accordance with the WHO recommendations.[20] The study of sperm quality included measuring the volume and pH of the ejaculate, the concentration of spermatozoa, the proportion of motile sperm of categories A and B, and LPO-AOD products.

In the seminal fluid of the examined men, the content of CD (primary oxidation products) and TBARS, end products of LPO, was determined by the methods of V. Gavrilov et al.[21,22] The level of α-tocopherol and retinol was estimated by the method of R. Ch.Chernyauskene et al., [23] TAA according to GI Klebanov et al. [24]

Statistical analysis was performed using STATISTICA 6.1 software (Stat-Soft Inc., USA). The normality of distribution of continuous variables was tested by one-sample Kolmogorov-Smirnov test. Baseline characteristics were summarized as mean±standard deviation (SD), median (Me) and interquartile range (IQR; 25th to 75th percentiles). Multiple comparisons were performed with one-way ANOVA with Tukey's pairwise comparisons. A value of $P<0.05$ was considered significant.

The study was carried out in compliance with Ethical Principles for Medical Research Involving Human Subjects, Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964, and amended by the 64th WMA General Assembly, Fortaleza, Brazil, October 2013. The study was approved by the Ethics Committee of the Scientific Center for Family Health and Human Reproduction Problems. Written informed consent was obtained from each patient.

Results and Discussion

Of the participants in the study, the group of somatically healthy men living in Irkutsk had the highest sperm count (mln per ml): 1.5 times more than men in Novosibirsk and 1.3 times more than men in Ulan-Ude. At the same time, in the group of men in Irkutsk, a significant increase in the concentration of α-tocopherol was revealed: 1.6 times more than the men in Ulan-Ude and 1.8 times more than the men in Novosibirsk (Table 1).

Table 1.

Indicators of spermatogenesis and LPO-AOD products in men from different cities of Siberia

Index	Irkutsk n=35 (1)	Novosibirsk n=50 (2)	Ulan-Ude n=40 (3)	*P*-value
Volume, ml	3.9±1.56 3.5 2.8;5.00	3.6±1.79 3.21 [2.28;4.41]	3.34±0.94 3.32 [2.82;3.74]	P=0.2757
Sperm count, mln/ml	82.9±43.04 80.6 [51.42;113.2]	54.04±47.7 40.28 [19.46;67.4]	63.32±38.97 51.56 [34.25;80.67]	P=0.0127 P_{1-2}=0.0093 P_{1-3}=0.1339 P_{2-3}=0.5786
Category A+B, %	51.59±17.42 52.00 [40.0;67.0]	48.26±29.67 47.95 [22.95;74.75]	57.69±15.96 55.96 [44.73;68.33]	P=0.1511
CD, µmol/l	1.28±0.82 1.26 [0.52;1.92]	1.25±0.97 0.92 [0.79;1.29]	1.26±0.68 1.16 [0.70;2.08]	P=0.9870
MDA, µmol/l	1.01±0.69 0.80 [0.71;1.12]	0.96±0.32 0.87 [0.69;1.17]	1.02±0.36 1.00 [0.71;1.23]	P=0.8047
TAA, stand. units	3.23±1.46 3.03 [2.32;4.94]	2.68±1.78 1.96 [1.41;3.80]	4.05±1.76 3.56 [2.94;5.54]	P=0.0010 P_{1-2}=0.3058 P_{1-3}=0.0948 P_{2-3}=0.0006
α-tocopherol, µmol/l	5.34±2.66 3.99 [3.56;6.82]	3.29±1.74 2.53 [2.01;4.47]	2.97±0.95 2.82 [2.15;3.62]	P=0.0000 P_{1-2}=0.0000 P_{1-3}=0.0000 P_{2-3}=0.6981

The data obtained confirm the theses of other researchers regarding the fact that the deficiency of α-tocopherol, the most important fat-soluble vitamin, exhibiting membrane-protective and antimutagenic activity, is primarily manifested by a reduced sperm count. Also, the deficiency of α-tocopherol

leads to a decrease in the volume of ejaculate, which was demonstrated in the groups of men in Novosibirsk and Ulan-Ude, in comparison with men in Irkutsk. Considering that the sperm membrane consists of lipids, and α-tocopherol plays a key role in the inhibition of LPO processes under the action of ROS, it can be assumed that the results obtained indicate the reliable functioning of the adaptive-protective mechanisms of the body in men living in Irkutsk.

Men in Ulan-Ude are characterized by a higher level of TAA: 1.5 times higher than men in Novosibirsk and 1.2 times higher than men in Irkutsk (Table 1). Considering that the group of men in Ulan-Ude has the lowest concentration of α-tocopherol, the high level of TAA, which characterizes the total activity of oxidation inhibitors and the body's ability to counteract the development of free radical reactions, can be explained both by the stress of the adaptive systems and reliable functioning of specialized enzymatic and non-enzymatic components of the AOD.

The complex process of spermatogenesis requires an optimal combination of various elements, including vitamins and trace elements. The physical, mental, and sexual health of a modern man depends on the quality of food, and the nature of and adherence to the diet. In particular, lack of attention to these factors is responsible for the disorders occurring in the reproductive systems of men, because if a sufficient amount of building material does not enter the body in a timely manner, then the cells begin to use substances that are similar in chemical properties, but inappropriate. The result of such a substitution can be the formation of sperm with reduced properties. The decrease in the indicators of spermatogenesis in men, demonstrated by studies in recent years, could be caused both by the increasing impact on the human body of damaging factors found in the environment, at work and in everyday life, and by the lack of vitamins and microelements in the diet of modern men.[1,7,8,10] According to a comprehensive assessment of nutrition in a number of regions of the world, there has been a sharp (2-3 times) decrease in the amount of food consumed by humans.[12] A decrease in the volume of food also reduces the amount of intake of necessary nutrients—minerals, vitamins, amino acids. At present, in developed countries the physical activity of the population has decreased, which indicates a decrease in energy consumption.[12]

The conducted studies of the quality of ejaculate and the characteristics of LPO processes in men from different cities of Siberia show that place of residence and ecological-geographical position are not the main reasons determining reproductive dysfunction, but can be important factors affecting the functioning of the reproductive system and determining the heterogeneity of male infertility in conditions of anthropogenic pressure.

Competing Interests

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

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Analysis of Pro- and Antioxidant Status in Women with Endocrine Infertility

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Abstract

The aim of our study was to identify the changes in the indicators of lipid peroxidation (LPO) and antioxidant defense (AOD) in women with endocrine infertility.

Methods and Results: The study included 358 women of reproductive age. Based on clinical and laboratory data, two groups were formed: the control group (CG) and the main group (MG). CG consisted of 61 healthy fertile women (mean age of 22±2.3 years). MG consisted of 297 women (mean age of 26±5.6 years) with endocrine infertility. The blood levels of antioxidant parameters (total antioxidant activity [TTA], SOD activity, α-tocopherol and retinol) and primary/secondary products of LPO (conjugated dienes [CD], ketodienes and conjugated trienes [KD-CT], and thiobarbituric acid reactants [TBARs]) were determined using spectrophotometric and fluorometric methods. The levels of sex hormones and cortisol were determined by radioimmunoassay and EIA.

In MG, we found a significant increase in the blood levels of total lipid, DB, CDs, KD-CT, and TBARs, compared to CG. The results of a comparative analysis of the AOD parameters showed a significant increase in the total AOA level by 31% in MG, while the levels of α-tocopherol and retinol significantly decreased by 22% and 15%, respectively, compared to CG. In women of MG, the serum level of prolactin increased by 2 times.

Conclusion: The results obtained reflect the adaptive and disadaptive mechanisms of LPO-AOD participation in the formation of female infertility of endocrine origin. The complex changes in the LPO-AOD system (increased levels of the substrates of oxidation and all intermediates of the LPO process amid falling values of the AOD parameters) indicate the presence of OS in women with endocrine infertility. (**International Journal of Biomedicine. 2020;10(4):373-377.**)

Key Words: women • lipid peroxidation • antioxidant defense • endocrine infertility

Abbreviations

AOD, antioxidant defense; **CDs,** conjugated dienes; **DB**, double bonds; **FSH**, follicle-stimulating hormone; **GSH,** reduced glutathione; **GSSG**, oxidized glutathione; **KD-CT**, ketodienes and conjugated trienes; **LH,** luteinizing hormone; **LPO**, lipid peroxidation; **OS**, oxidative stress; **PRL**, prolactin; **ROS**, reactive oxygen species; **SOD**, superoxide dismutase; **TAA**, total antioxidant activity; **TBARs**, thiobarbituric acid reactants; **WHO**, world health organization; **17-OH-Pg**, 17-OH-progesterone; **GSH**, reduced glutathione; **GSSG**, oxidized glutathione.

Introduction

The non-specific biochemical processes that occur at the cellular level and determine the reactivity of the body are essential in the pathogenesis of neuroendocrine diseases of the reproductive system.[1-7] It is known that the reactions of lipid peroxidation (LPO) and antioxidant defense (AOD) provide cellular homeostasis at the optimal level. In disregulatory states, as a result of the LPO activation, a violation of the pro-oxidant-antioxidant balance occurs, which indicates the development of oxidative stress (OS).[8,9]

One of the most powerful factors that damage cell membranes is the formation of free radicals, which cause chain reactions of lipid oxidation in the cell. Especially

susceptible to free radical oxidation are membrane phospholipids unsaturated fatty acids. Excessive activity of free radical lipid oxidation processes is prevented by a special AOD system. AOD plays an important role in the body's protective and adaptive responses. The study of the balance of indicators of the free radical lipid oxidation processes and AOD in various pathological processes allows us to assess patients' reserve capabilities.[10,11]

To fully understand the nature of peroxidation processes in reproductive disorders, and when to choose tactics for rational correction of their possible damaging effects, a comprehensive examination is necessary, including assessment of both the initial and final LPO products, as well as the level of factors that provide defense from possible damage to the cell regulatory apparatus by intermediates of LPO.[12] Women's reproductive health protection is one of the crucial tasks of healthcare. Despite the advances in modern medicine and the development and implementation of new reproductive technologies, the number of infertile couples continues to increase and, according to the literature, the frequency of infertility in various countries of the world varies from 8% to 21%, and the endocrine female infertility accounts for about 30%-40%.[13,14]

Despite extensive study of the main mechanisms of pathogenesis, and of factors that prevent the development of the most common clinical and pathogenetic variants of female endocrine infertility and reproductive disorders, the background of hormone-dependent gynecological diseases is still not clear. It concerns functional relationships between the processes of LPO and neuroendocrine regulation of reproductive disorders, which is important for women with endocrine infertility.[15,16]

The aim of our study was to identify the changes in the indicators of LPO and AOD in women with endocrine infertility.

Materials and Methods

The study was conducted in accordance with ethical principles of the WMA Declaration of Helsinki (1964, ed. 2013) and approved by the Ethics Committee of Scientific Centre for Family Health and Human Reproduction Problems. Written informed consent was obtained from the patient/ parent/guardian/ relative of each patient.

To evaluate the parameters of the LPO-AOD system, 358 women of reproductive age were selected. When assigning women to a particular category (fertile or infertile), the WHO classification of fertility was used: fertile (women who had a pregnancy during the current year), presumably fertile (women who had a history of pregnancy more than 1 year ago), primarily infertile (women who did not have a history of pregnancy, despite regular sexual contact during the year and provided that no contraceptive methods were used), secondarily infertile (infertile women with a history of pregnancy), women with unknown fertility (women with no history of pregnancy due to the use of contraceptive methods, and/or with irregular sexual contact). Women with unknown fertility were not included in the study.

Based on clinical and laboratory data, taking into account the accepted criteria, two groups were formed: the control group (CG) and the main group (MG). CG consisted of 61 healthy fertile women (mean age of 22±2.3 years).

The criteria for inclusion in the CG: pregnancy that ended in childbirth within the last 2 years, absence of neuroendocrine disorders, regular menstrual cycle, and absence of severe somatic pathology. MG consisted of 297 infertile women (mean age of 26±5.6 years) with the following nosological forms and syndromes: hypothalamic syndrome (n=108), hyperprolactinemia (n=60), congenital adrenal cortex dysfunction(n=32), and dyshormonal diseases of the mammary glands(n=97). Women of all infertile subgroups were comparable in age, reproductive history, and duration of infertility.

Blood samples (5 ml) were collected from the ulnar vein in standard vacuum tubes with EDTA in the morning after night fasting. The erythrocyte population was separated from the other blood components by centrifugation at 1500 *g* for 5 min, at 4°C. The erythrocyte pellet was washed 3 times with a 0.9% (wt/vol) NaCl solution. Aliquots of EDTA anticoagulated plasma and washed erythrocytes were used immediately or kept frozen at -40°C, not exceeding one month. Total lipids were measured by colorimetric kit assay (BIOLA-TEST, PLIVA - Lachema a.s., Czech Republic).

The intensity of LPO-AOD processes was estimated by plasma concentrations of antioxidant parameters (TAA, SOD activity, α-tocopherol and retinol) and primary/secondary products of LPO (CDs, KD-CT, and TBARS).[17,18] The blood plasma TAA level was detected photometrically.[19] TBARs levels, SOD activity in hemolysate and α-tocopherol and retinol levels in plasma were detected by fluorometry.[20] The content of GSH and GSSG was determined by P.Y. Hissin, R. Hilf (1976).[21] The SOD activity in the erythrocytes was determined by H.P. Misra and I. Fridovich.[22] The measurements were conducted with a Shimadzu RF-1501 spectrophotometer (Japan) consisting of two blocks: a UV-1650PC spectrophotometer and a RF-1501 spectrofluorimeter.

The levels of LH, FSH, PRL in the blood were detected by radioimmunoassay with an Immunotest Analyzer (Russia) using commercial kits from Dias (Russia). The level of cortisol was determined by EIA with EL 808 analyzer (USA) using test system "ALKOR-BIO" (Russia), 17-OH-progesterone a COBOS analyzer (USA) using kits from DRG ELISAS (USA).

Statistical analysis was performed using the Statistica 6.1 software package (Stat-Soft Inc., USA). The normality of distribution of continuous variables was tested by the Kolmogorov-Smirnov test with the Lilliefors correction and Shapiro-Wilk test. For descriptive analysis, results are presented as mean±standard deviation (SD). 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. A probability value of $P<0.05$ was considered statistically significant.

Results

In MG, total lipid concentrations increased by 8% and DB - by 13%, compared to CG. Activation of the LPO process, leading to the accumulation of the toxic products, was characterized by an increase in the levels of the lipid oxidation primary and secondary products [CDs – by 20% ($p<0.05$), KD-CT – by 29% ($p<0.05$)], as well as a significant increase in the level of TBARs (Fig.1).

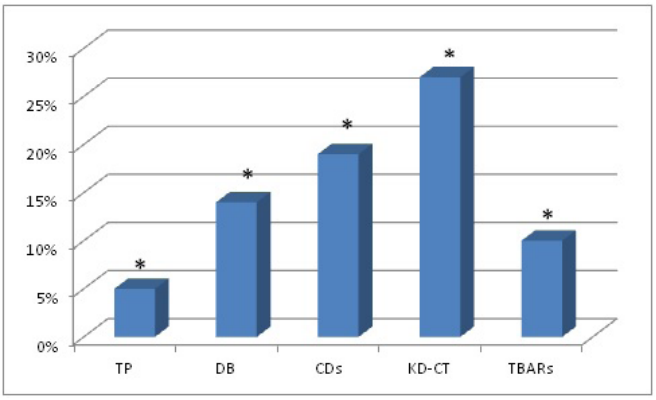

Fig. 1. *The blood levels of oxidation substrates and LPO products in study groups. (0 – the level of the control group indicators, taken as 100%; * - P <0.05).*

The results of a comparative analysis of the AOD parameters showed a significant increase in the total AOA level by 31% in MG, while the levels of α-tocopherol and retinol significantly decreased by 22% and 15%, respectively, compared to CG. The SOD activity was 6% lower than in CGThe blood level of GSSG increased by 5% and the GSH/GSSG ratio decreased insignificantly by 5% in MG, compared to CG (Fig.2).

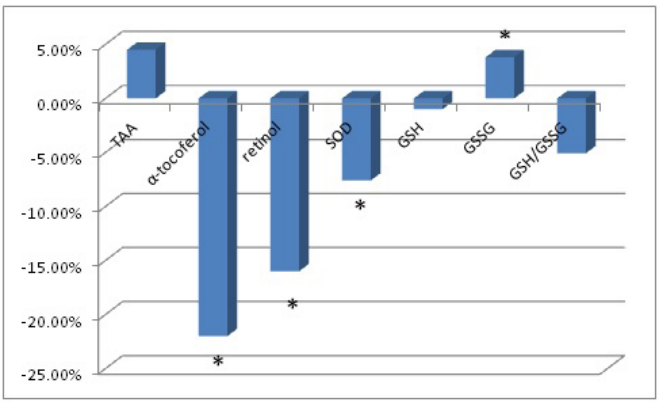

Fig. 2. *The blood levels of AOD indicators in study groups. (0 – the level of the control group indicators, taken as 100%; * - P <0.05).*

In women of MG, the serum level of PRL increased by 2 times, and the LH/FSH ratio was 19% higher than in CG; there were no significant differences in the levels of LH and FSH between groups (Fig.3). We found also a 17% increase in the levels of cortisol and a 19% increase in 17-OH-progesterone in MG, compared to CG. No significant differences in the serum levels of testosterone, thyroid and thyroid-stimulating hormones were revealed between study groups.

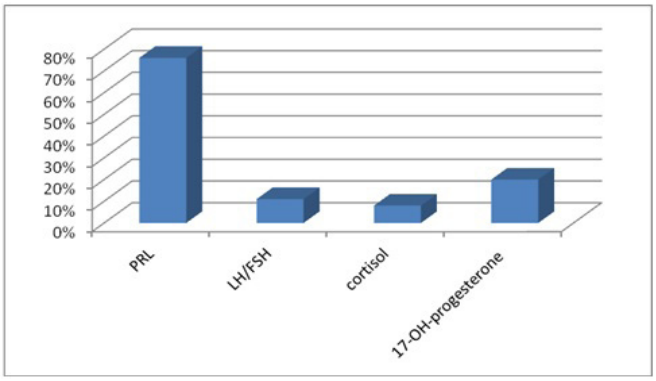

Fig. 3. *The blood levels of sex hormones and cortisol in study groups. (0 – the level of the control group indicators, taken as 100%; * - P <0.05).*

Biochemical studies of human reproductive function and its disorders in recent decades have been characterized by the development of "cost-effective" universal technologies that allow us to give an integral assessment and outline some sanogenetic ways to correct disorders in reproductive function. It is necessary to highlight the research concerning the polyvalent function of LPO and AOD factors,[23] which prevent the development of OS and positively affect the complex treatment of endocrine forms of female infertility.[24,25]

The increased intensity of LPO, as a possible result of neurohumoral shifts, in many cases is either a consequence or cause of certain pathological changes in cells and tissues.[26,27]

The concentration of highly reactive LPO products is controlled by a multicomponent system of AOD, formed during evolution, that prevents the formation of OS.[28]

In a healthy subject, the actions of antioxidants are closely related to each other and are clearly balanced. Violation of the ratio of enzymatic and non-enzymatic components of AOD can cause additional activation in LPO processes and depletion of antioxidant reserves, leading to the development of OS. In our study, the analysis of the antioxidant system factors demonstrated either reduced concentration (α-tocopherol, retinol) and low activity (superoxide dismutase), or almost unchanged levels (glutathione system).

The decrease of retinol concentration in women with endocrine infertility, compared to women of CG, deserves special attention. This fact should be attributed not so much to the lower antioxidant status of the blood plasma of these patients, but to the fact that during its oxidation vitamin A is converted to retinoic acid.[29] Retinoic acid is currently considered as a lipophilic hormone that interacts with the nucleus of target cells like steroid hormones. The resulting complex binds to certain parts of the DNA and stimulates gene transcription. Proteins formed as a result of gene stimulation under the influence of retinoic acid affect the growth, differentiation, and regeneration of tissues. In this regard, retinol can be considered not only a

direct-acting antioxidant, responsible together with tocopherols for the "quenching" of peroxide radicals, but also a factor that has an antioxidant-mediated effect through the hormonal regulation of systemic metabolism and the functioning of many organs and systems.[30] This possibility is indicated by the results of studies that have shown retinoic acid binds to the receptors of vessels of smooth muscles, whose nuclei are constantly in the "stand by" mode, activating the corresponding regions of DNA with the formation of adrenomedullin, which induces a variety of systemic effects.[31]

Of considerable interest is also the fact that in the group of patients with endocrine infertility, prolactin content is twice as high as in healthy women. According to the literature data on the effect of prolactin on lipid metabolism, women have a positive correlation between the content of triacylglycerols and prolactin.[32] The results suggest the possibility of increasing the activity of liposynthetic processes in the liver, a possibility that is also evidenced by data on an increase in the concentration of free fatty acids, cholesterol, and triacylglycerols in animals after intraperitoneal administration of prolactin.[33] Prolactin inhibits human lipoprotein lipase activity in white adipose tissue,[34] thereby maintaining a high concentration of lipids in the blood. In this regard, a paradoxical metabolic situation arises: in the presence of absorptive lipemia, triacylglycerols are not attacked by the prolactin-blocked lipoprotein lipase and do not replenish the fat depot. The formation of fatty acids de novo, and lipids from them, is prevented by the depression of malonyl-CoA synthetase, which can occur at the level of apoenzyme, coenzymes, or cofactors. At the same time, the residues of unsaturated fatty acids remaining in the blood for a long time in the composition of LDL are very likely to undergo lipid peroxidation, which we observe when the concentration of prolactin increases.

Thus, our results reflect the adaptive and disadaptive mechanisms of LPO-AOD participation in the formation of female infertility of endocrine origin. Taking into account the complex changes in the LPO-AOD system, indicating the presence of OS (increased levels of the substrates of oxidation and all intermediates of the LPO process amid falling values of the AOD parameters), we recommend the differentiated complex treatment and preventive measures aimed at normalizing the of LPO-AOD balance in women with endocrine infertility.

Competing Interests

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Radiology

INTERNATIONAL JOURNAL OF BIOMEDICINE

Mammographic Density Errors during Interpretation of Breast Examinations

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Abstract

Background: This article discusses errors that may occur when radiologists deal with mammographic density (MD) and present their performance analysis in Russia.

Methods and Results: The authors of this report have analyzed 24,485 mammographic reports for 20,000 female patients (aged 29-85 years) that were made in various healthcare facilities in the Russian Federation. In 249(1.7%) mammographic exam reports, we observed a wrong MD interpretation.

Conclusion: Diagnostic errors during mammographic examination occur because the radiologists lack theoretical knowledge about MD. This lack, in turn, results in a decrease in the quality of medical care provided for female patients and an increase in the number of diagnostic iatrogeny. (**International Journal of Biomedicine. 2020;10(4):378-381.**)

Key Words: mammographic density • iatrogeny • error • breast cancer

Abbreviations

MD, mammographic density; **BC**, breast cancer.

Introduction

Despite the fact that medicine is constantly developing, and there are many scientific works devoted to the diagnosis and treatment of breast cancer (BC), the incidence and mortality rates of this disease are among the highest of all the cancer types.[1-3] Like any other pathologic process, a BC has its own risk factors. Many scientists believe that mammographic density (MD) is the key BC predictor.[4-6] Various sources state that according to the mammogram results, more than half of female patients under 50 have high MD values.[7] It is worth mentioning that the mammograms that display a high and extreme MD are often hard to interpret.[8] On the one hand, there is a risk of obtaining a false-negative result because the diagnostic accuracy of mammography decreases; on the other hand, a risk of a false-positive due to the tissue architecture superposition effect.[9,10] That is why the MD values are vital for determining the risk of breast cancer development. They should be under control throughout a patient's life.[11] The scientists have proposed both quantitative and qualitative approaches to assessing MD, but the American College of Radiology (ACR) has strongly recommended using the latter one since 2013.[12-14] If MD is classified as "C" or "D," the female patients are strongly recommended to undergo supplementary screenings; in most cases, this is an ultrasound scan, no matter whether they have pathologic changes or not.[15] However, due to certain subjectivity of classifications, there is some variability in mammographic pattern interpretation. This often results in miscategorization of MD and, consequently, in errors in further patient follow-up.

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Materials and Methods

The authors of this report have analyzed 24,485 mammographic reports for 20,000 female patients (aged 29-85 years) that were made in various healthcare facilities in the Russian Federation in the period of 2008-2020, in order to assess the quality of work of Russian radiologists with regard to MD interpretation.

Results and Discussion

The MD category has been stated in 14,509(59.4%) cases. In 9,916 (40.6%) reports, there was no data about the MD category. If we speak about 14,509 cases, where the mammographic density has been stated, it is worth mentioning that in 6,645 (45.8%) cases the radiologists resorted to the outdated method of MD detection, which is based on the calculation of the percentage ratio of fat and fibroglandular components (Wolfe, 2003). Since 2013, the ACR has been taking into account the fact that the pathologic area can disguise itself as dense structures of a breast. Besides, Roman numerals (I-IV) that used to denote the MD grades were replaced by letters (A, B, C, D). As the research data embraced the period of 2008- 2020, we did not consider a percentage ratio method as a completely improper one. We have divided the mammographic reports into 2 groups, depending on the assessment systems for MD of breast tissue applied. The graph is shown in Fig. 1.

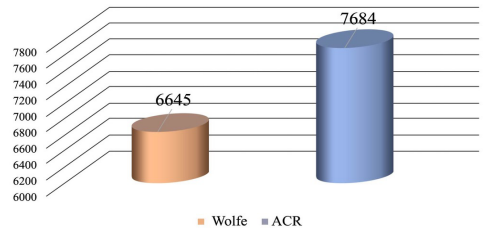

Fig. 1. *The distribution of cases, depending on the employed method of evaluation and interpretation of MD (n=14,509)*

In 249 (1.7%) mammographic exam reports, we observed a wrong MD interpretation. The correlation between errors in MD detection and the total amount of categorized cases is shown in Fig.2.

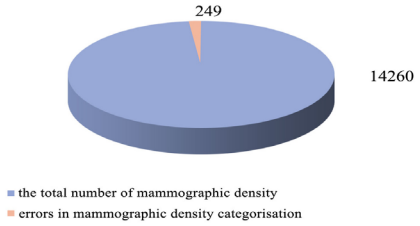

Fig. 2. *The error rate in MD of mammary gland tissue, if the density category is stated (n=14,509).*

Table 1 contains the cases of the wrong MD interpretations (249=100%).

Table 1.

The distribution of the misassigned categories of MD (n=249)

	MD according to radiologists' reports	
Category	Abs.	(%)
ACR BI-RADS A	14	5.6
ACR BI-RADS B	151	60.6
ACR BI-RADS C	66	26.5
ACR BI-RADS D	18	7.2

We have divided them into the following categories:

1) 14 (5.6%) cases were classified as ACR BI-RADS A, while mammograms have shown that there is a sufficient amount of fibroglandular tissue.

2) 151 (60.6%) cases were miscategorized as ACR BI-RADS B instead of A (n=104) or C (n=47) (Fig.3).

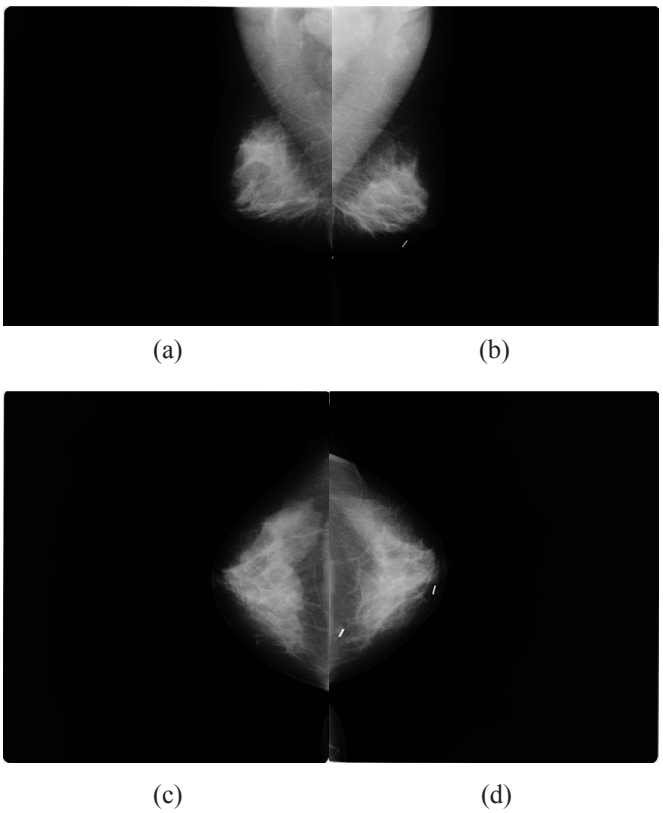

Fig. 3. *A 49-year-old woman. Mammograms in 2 standard planes: a, c – mammograms of a right breast in inclined and straight planes; b, d – mammograms of a left breast in inclined and straight plane. In the radiological conclusion, MD of breast tissue is identified as ACR BI-RADS B; the female patient is supposed to undergo a mammographic exam one more time in 2 years. The category is stated incorrectly, MD of breast tissue of this patient corresponds to ACR BI-RADS C. That means that she should make an ultrasound scan immediately.*

3) Heterogeneous MD (ACR BI-RADS C) was misinterpreted in 66 (26.5%) cases, while the exams of 52 female patients had to be interpreted as ACR BI-RADS B (Fig. 4) and 14 as ACR BI-RADS D.

4) Errors with ACR BI-RADS D were quite rare. There were only 18 (7.2%) reports with ACR BI-RADS D, while the breast did not entirely consist of fibro-glandular tissue. There were numerous heterogeneous areas of a dense structure (ACR-BI-RADS C).

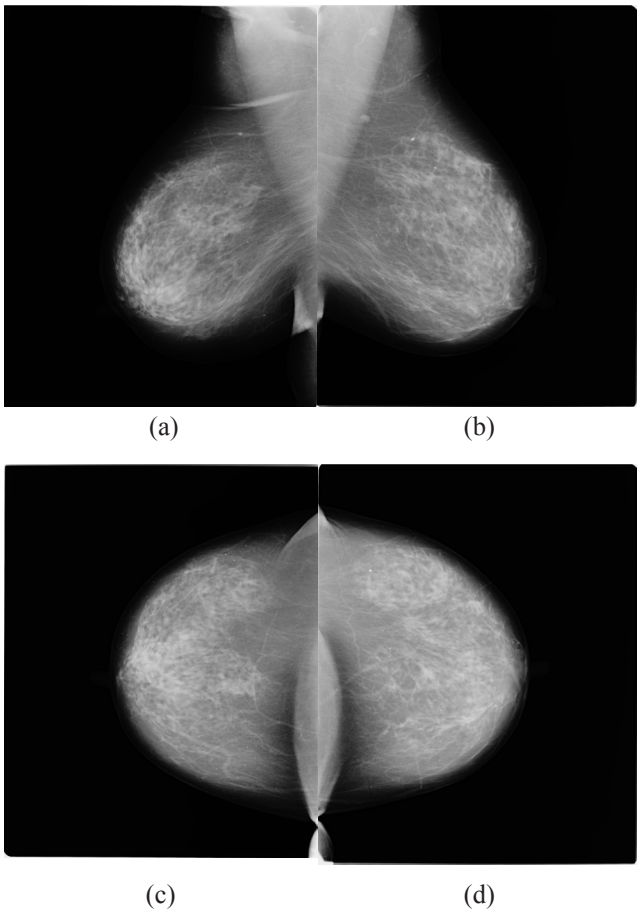

Fig. 4. *A 53-year-old woman. Mammograms in 2 standard planes: a, c – mammograms of a right breast in inclined and straight planes; b, d – mammograms of a left breast in inclined and straight planes. In the radiological report, MD of breast tissue is identified as ACR BI-RADS C; the radiologist has recommended to make an ultrasound scan. The category is stated incorrectly, MD of breast tissue of this patient corresponds to ACR BI-RADS B. That means that the patient has to undergo a routine and control mammographic exam with respect to her age.*

After analyzing the results of the conducted research, we can conclude that Russian radiologists register MD in less than a half of all the mammographic exam reports. If we speak about cases, when ACR BI-RADS categories have been identified, the most crucial diagnostic errors were B miscategorizations, when actually there were heterogeneous dense structures, and incorrectly stated C category for breasts with sporadic areas of fibroglandular tissue. In the first case, the patients won't undergo an ultrasonic scan, which is obligatory and indicated for patients with high and extreme MD, and this can result in false-negative diagnostic results. In the second case, on the contrary, an ultrasonic scan will complicate a diagnostic path and may lead to stressful situations for the patients.

In conclusion, when radiologists fail to state the category of the MD, or misidentify it, this often results in an increase in iatrogenic injuries of the breast, as well as in a decrease in the quality of medical care provided for patients that leads to late detection of malignant activity in mammary glands.

Competing Interests

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Radiology

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Assessment of Brain Lesions in Type 2 Diabetes Mellitus and Hypertension using Magnetic Resonance Imaging

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Abstract

Background: Type 2 diabetes mellitus (T2DM) and hypertension (HTN) are risk factors for the spectrum of brain lesions. In this paper, we studied the impact of T2DM and HTN on the incidence of several brain lesions diagnosed with magnetic resonance imaging (MRI).

***Methods and Results*:** This retrospective, single-center study was conducted at Royal Care International Hospital (Khartoum, Sudan) from January 2016 to December 2016 and included 80 patients (40 male and 40 female, aged between 20 years and 90 years) with suspected brain disorders. MRI brain examinations were conducted on a 1.5 Tesla MRI system (Toshiba Medical Systems, Tokyo, Japan). The following sequences were analyzed: T1-weighted imaging (T1WI), T2-weighted imaging (T2WI), fluid-attenuated inversion recovery (FLAIR), and diffusion-weighted imaging (DWI). Brain lesions were characterized by magnetic imaging spectroscopy and histopathological analysis. Binary logistic regression analysis was used to establish a mathematical model of the relationship between T2DM/HTN and the prevalence of brain lesions.

Among 80 patients, HTN, T2DM, and the combination of T2DM and HTN were identified in 18(22.5%), 9(11.2%), and 11(13.8%) patients, respectively. Brain lesions were found in 48(60%) patients and were most prevalent in the age group of 66-80 years. The brain lesions included ischemic brain infarction (22.5%), brain tumors (11.2%), cerebral hemorrhages (6.2%), brain atrophy (1.2 %), ischemic brain infarction with brain atrophy (16.2%), and brain metastases (2.5%). Regression analysis showed that HTN and T2DM were associated with significantly higher ORs for brain lesions ([OR=2.459, 95% CI: 1.673–3.614, P<0.001] and [OR=1.507, 95% CI: 1.067–2.128, P=0.042], respectively). HTN was associated with significantly higher OR for ischemic brain infarction (OR=7.404, 95% CI: 2.600–21.081, P<0.001).

Conclusion: The study showed a significant interaction between HTN and T2DM on the prevalence of brain lesions, especially ischemic brain infarction and brain atrophy.(**International Journal of Biomedicine. 2020;10(4):382-386.**)

Key Words: brain lesions • MRI • ischemic brain infarction • diabetes • hypertension

Abbreviations

BA, brain atrophy**; CH**, cerebral hemorrhages; **DWI**, diffusion-weighted imaging; **FLAIR**, fluid-attenuated inversion recovery; **HTN**, hypertension; **IBI**, ischemic brain infarction; **MRI**, magnetic resonance imaging; **T2DM**, type 2 diabetes mellitus.

Introduction

Type 2 diabetes mellitus (T2DM) and hypertension (HTN) are common risk factors associated with a spectrum of brain lesions such as ischemic brain infarcts (IBI), neurodegenerative outcomes, brain atrophy (BA), late-life cognitive impairment, and others.[1-4]

Magnetic resonance imaging (MRI) is widely used in diagnosing brain ischemic lesions, intracranial tumors, brain metastases, and other lesions. With recent advances in MRI technology, ischemic lesions can be identified with high accuracy using diffusion-weighted image (88%-100% sensitivity and 95%-100% specificity).[5] New diagnostic techniques, such as dynamic color mapping, diffusion-weighted imaging, diffusion

tensor imaging, perfusion-weighted imaging, magnetic resonance spectroscopy, and functional MRI allow us to obtain detailed information about brain lesions.[6-8]

The aim of our study was to study the impact of T2DM and HTN on the incidence of several brain lesions diagnosed with MRI.

Materials and Methods

This retrospective, single-center study was conducted at Royal Care International Hospital (Khartoum, Sudan) from January 2016 to December 2016 and included 80 patients (40 male and 40 female, aged between 20 years and 90 years) with suspected brain disorders. Among 80 patients, HTN, T2DM, and the combination of T2DM and HTN were identified in 18(22.5%), 9(11.2%), and 11(13.8%) patients, respectively.

HTN was diagnosed when a person's systolic blood pressure (SBP) was ≥140 mmHg and/or their diastolic blood pressure (DBP) was ≥90 mmHg following repeated examination.[9] Diabetes was diagnosed when the patient has a fasting blood glucose level of 126 mg per dL (7.0 mmol per L) or greater on two separate occasions.[10]

The data collection sheets completed for each subject included the following variables: sex, age, clinical history of HTN and T2DM, and MRI findings.

This study was approved by the ethics committee of the Royal Care International Hospital. Written informed consent was obtained from each patient.

MRI brain examinations were conducted on a 1.5 Tesla MRI system (Toshiba Medical Systems, Tokyo, Japan). Each patient was scanned supine on the examination couch with his or her head within the head coil. Images were obtained in a plane orthogonal to the long axis of the hippocampus; this plane is orientated parallel to the brainstem. The following sequences (slice thickness/interslice distance of 5mm/1.5mm) were analyzed: T1-weighted imaging (T1WI), T2-weighted imaging (T2WI), fluid-attenuated inversion recovery (FLAIR), and diffusion-weighted imaging (DWI). Brain lesions were characterized by magnetic imaging spectroscopy and histopathological analysis.

Statistical analysis was performed using IBM SPSS Statistics 23. Binary logistic regression analysis was used to establish a mathematical model of the relationship between T2DM/HTN and the prevalence of brain lesions. Odd ratios (OR) and their 95% confidence intervals (95%CI) were calculated. A probability value of $P<0.05$ was considered statistically significant.

Results and Discussion

The demographic, clinical characteristics and medical history of study participants are presented in Table 1. Among 80 patients, HTN, T2DM, and the combination of T2DM and HTN were identified in 18(22.5%), 9(11.2%), and 11(13.8%) patients, respectively. Brain lesions were identified in 48(60%) patients (Table 1) and were most prevalent in the age group of 66-80 years. The brain lesions included ischemic brain infarction (IBI) (22.5%), brain tumors (11.2%), cerebral hemorrhages (CH) (6.2%), brain atrophy (BA) (1.2 %), IBI with BA (16.2%), and brain metastases (2.5%). IBI with and without BA was found in 16.2% and 22.5% cases, respectively, (Fig.1 and Table 2) and accounted for 27.1% and 37.5%, respectively, of all brain lesions. Brain tumors accounted for 18.8% of all brain lesions, whereas cerebral hemorrhage for 10.4%. The other brain lesions were less frequent. IBI was most common in the age groups of 51-65 and 66-80, whereas IBI and BA were most frequent in the age group of 66-80 years (Table 2). The prevalence of brain tumors was most frequently found in the age group of 36-50 years. Figure 2 shows the distribution of brain lesions between males and females. It was observed that IBI and IBI+BA were higher among males than among females (10 vs. 8 and 9 vs. 4, respectively). It was found that IBI+BA was prevalent (63.6%) in patients with T2DM and HTN (Table 3).

Table 1:

The demographic, clinical characteristics and medical history of study participants

Variable	Absolute number	Percent, %
Males	40	50
Females	40	50
Age groups		
20-35 years	19	16.7
36-50 years	18	15.8
51-65 years	16	14.0
66-80 years	23	20.2
81-90 years	4	3.5
Clinical history		
None	42	52.5
T2DM	9	11.2
HTN	18	22.5
T2DM+HTN	11	13.8
MRI Diagnosis of brain lesions		
Yes	48	60.0
No	32	40.0

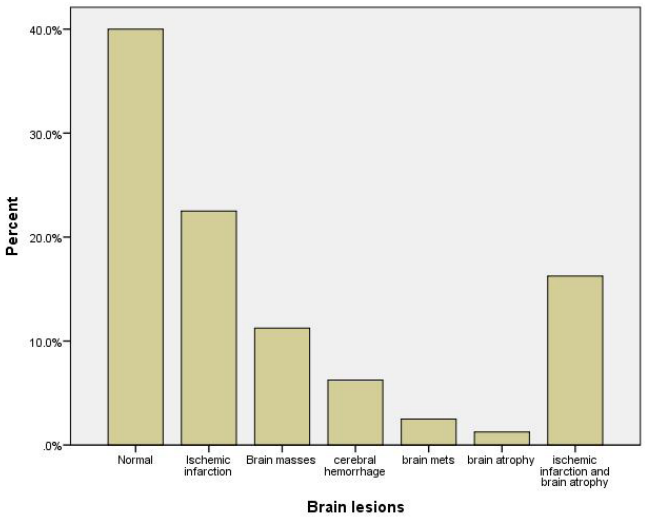

Fig. 1. *Distribution of spectrum brain lesions in the study sample.*

Table2.

Incidence of brain lesions in different age groups

Age groups	No brain lesions	IBI	Brain tumors	CH	Brain Mets	BA	IBI and BA
20-35 yrs (n=19)	15 (78.9%)	2 (10.5%)	1 (5.3%)	1 (5.3%)	-	-	-
36-50 yrs (n=18)	12 (66.7%)	2 (11.1%)	4 (22.2%)	-	-	-	-
51-65 yrs (n=16)	3 (18.8%)	8 (50.0%)	1 (6.2%)	1 (6.2%)	2 (12.5%)	-	1 (6.2%)
66-80 yrs (n=23)	2 (8.7%)	6 (26.1%)	3 (13.0%)	3 (13.0%)	-	1 (4.3%)	8 (34.8%)
81-90 yrs (n=4)	-	-	-	-	--	-	4 (100%)
Total (n=80)	32 (40.0%)	18 (22.5%)	9 (11.2%)	5 (6.2%)	2 (2.5%)	1 (1.2%)	13 (16.2%)

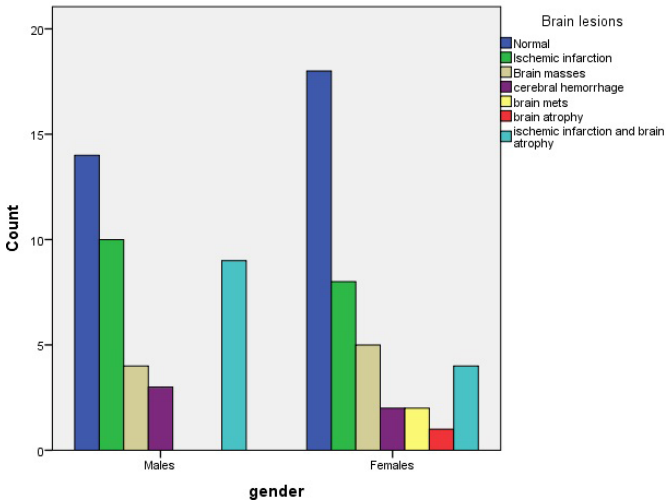

Fig. 2. *Distribution of brain lesions according to gender*

Table 3.

Brain lesions in the study participants

Clinical history	Brain lesions						
	No brain lesions	IBI	Brain tumors	CH	Brain Mets	BA	IBI and BA
No history of T2DM and HTN	25 (59.5%)	7 (16.7%)	5 (11.9%)	1 (2.4%)	2 (4.8%)	-	2 (4.8%)
T2DM (n=9)	4 (44.4%)	3 (33.3%)	1 (11.1%)	1 (11.1%)	-	-	-
HTN (n=18)	2 (11.1%)	7 (38.9%)	2 (11.1%)	2 (11.1%)	-	1 (5.6%)	4 (22.2%)
T2DM and HTN (n=11)	1 (9.1%)	1 (9.1%)	1 (9.1%)	1 (9.1%)	-	-	7 (63.6%)
Total (n=80)	32 (40.0%)	18 (22.5%)	9 (11.2%)	5 (6.2%)	2 (2.5%)	1 (1.2%)	13 (16.2%)

T2DM was associated with a significantly higher OR for cerebral hemorrhage, although the relationship was statistically insignificant (P=0.22) (Fig.3). The associations between brain tumors and T2DM or HTN were of no significance (P>0.05) (Fig.4).

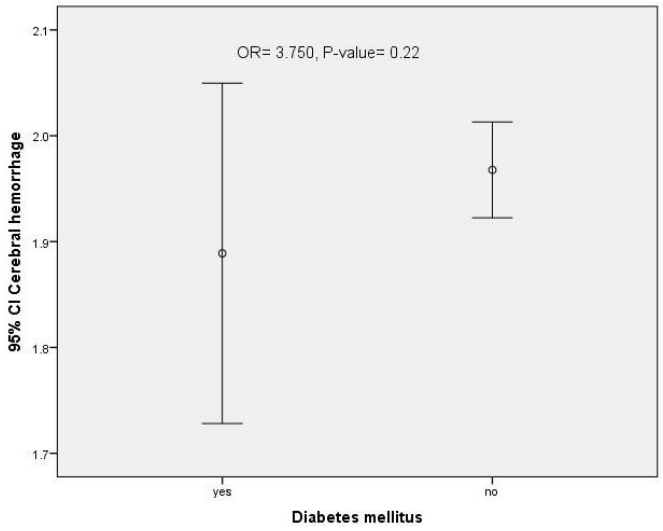

Fig. 3. *Association of T2DM with cerebral hemorrhages*

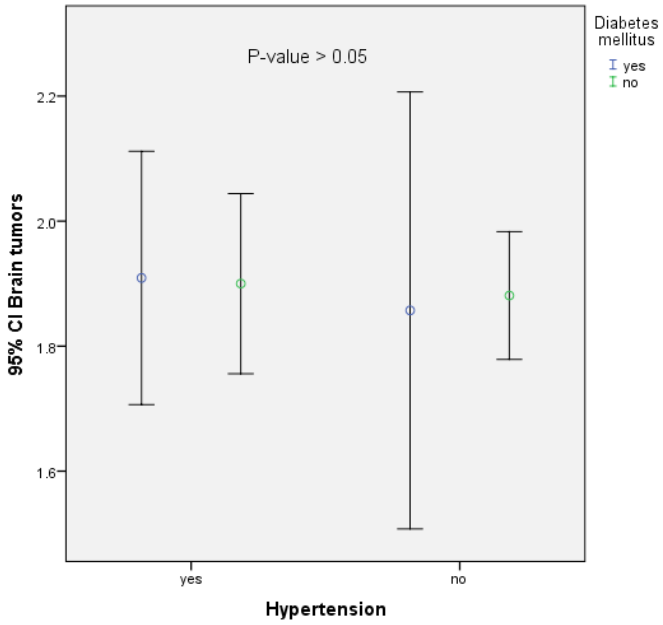

Fig. 4. *Association of T2DM and HTN with brain tumours*

Regression analysis showed that HTN and T2DM were associated with significantly higher ORs for brain lesions ([OR=2.459, 95% CI: 1.673–3.614, P<0.001], [OR=1.507, 95% CI: 1.067–2.128, P= 0.042] (Table 4). Table 5 summarizes the associated factors of ischemic brain infarction. HTN was associated with significantly higher OR for ischemic brain infarction (OR=7.404, 95% CI: 2.600–21.081, P<0.001), while the associations between the risk of ischemic brain infarction and sex or diabetes were of no significance.

T2DM and HTN are potent risk factors for cerebrovascular disease. Brain ischemic lesions are frequently seen on brain MRI especially in the elderly population. Hypertension and

T2DM were reported to be strongly associated with risks of vascular brain lesions, neurodegeneration, IBI, and BA.[11-15] The evidence for the relation between blood pressure and brain atrophy is less clear.[12] Some studies showing that high midlife blood pressure is related to BA later in life,[16-18] while others show that in older individuals, especially low blood pressure levels lead to an increased risk for BA.[19,20]

Table 4.

Binary logistic regression analysis of associated factors for brain lesions

Variable	B	SE	χ^2	Sig.	OR	95%CI
Gender	-0.077	0.635	0.015	0.904	0.926	0.267–3.216
Age	0.075	0.022	12.160	0.001	1.078	1.033–1.124
HTN	-1.565	0.802	22.312	< 001	2.459	1.673–3.614
T2DM	0.109	0.839	3.908	0.042	1.507	1.067–2.128

Table 5.

Binary logistic regression analysis of associated factors for brain ischemic infarction

Variable	B	SE	χ^2	Sig.	OR	95%CI
Gender	0.494	0.601	0.675	0.411	1.639	0.504–5.327
Age	-0.065	0.021	9.457	0.002	0.937	0.900–0.977
HTN	2.002	0.534	14.060	<0.001	7.404	2.600–21.081
T2DM	1.032	0.634	2.646	0.104	2.806	0.810 –9.723

The current study supports the hypothesis that T2DM and HTN increased the risk of various brain lesions. IBI and BA were the most prevalent lesions in the study sample with HTN and T2DM. Our results are in agreement with the data obtained by Roberts et al. [21] Moran et al. reported that T2DM was associated with more cerebral infarcts and lower total gray, white, and hippocampal volumes.[22]

The mechanisms by which T2DM and HTN cause brain lesions are disputed. They may primarily target blood vessel structure and function to cause vascular damage or may interact at the cellular level with neurons or synapses that affect neurodegenerative processes and promote brain neurodegeneration.[21,23,24]

As we age, structural changes occur throughout the brain. Such common brain changes include global atrophy, white matter injury, small-vessel ischemia, and microhemorrhages. (25-29) These changes are more frequent and more severe in HTN and T2DM. The present study revealed that the incidence of brain lesions, especially brain atrophy, increased with age. Our results are consistent with T. Gu et al.[30] who found that age and the number of cardiovascular risk factors are independently associated with the brain atrophy and lesion index score.

Accumulating evidence suggests that a history of diabetes may be involved in the occurrence of various types of cancer.[31-34] The findings of the meta-analysis performed by Tong et al [35] indicate that diabetic individuals have a similar risk of brain tumors as non-diabetic individuals. However, a significant positive correlation between the risk of brain tumors and diabetes mellitus was revealed in females, but not in males. Our study found that DM and HTN were not significantly associated with the incidence rates for brain tumors.

In conclusion, HTN and T2DM are associated with advanced brain damage. The current study showed a significant interaction between HTN and T2DM on the prevalence of brain lesions, especially ischemic brain infarcts and brain atrophy.

Competing Interests

The authors declare that they have no competing interests.

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Evaluation of Traumatic Knee and Shoulder Joint Ligaments with MRI among Adult Sudanese Patients

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Abstract

Background: *M*agnetic resonance imaging (MRI) is currently regarded as the reference standard for the diagnosis and evaluation of internal derangements of the knee and shoulder girdle. This study aimed to evaluate traumatic injuries of the knee and shoulder joint ligaments by MRI and classify the most common ligament injuries.

Methods and Results: This study included 50 patients, who were presented in the Radiology Department of Modern Medical Center in Khartoum, in the period from October 2019 to January 2020. The sample was divided into two groups: shoulder joint group (Group 1, n=30) with an age range between 16 and 74 years and knee joint group (Group 2, n=20) with an age range between 16 and 77 years. The age group most affected with shoulder (46.7%) and knee joint (25%) injuries was 46-65 years. The injuries of the shoulder (40%) and knee joints (55%) were common in patients with body weight ranging from 71-80 kg and >81 kg, respectively. Different grades of partial meniscus tear of both shoulder and knee joints were noted as Grades 1 and 2 in 8.7% of cases, Grade 2 - 13%, Grades 2 and 3 - 34.8%, and Grade 3 - 30.4%. There were incidences of 27.3%, 0%, 54.5%, 15.20, 0%, and 3% for anterior cruciate ligament, posterior cruciate ligament, medial meniscus C-shape (M Me C), lateral meniscus C-shape (L Me C), medial collateral ligament, and lateral collateral ligament tears in the knee joints of the affected patients.

Conclusion: MRI revealed that injuries to the shoulder and knee joints were common in patients with body weight ranging from 71-80 kg and >81 kg, respectively. Different grades of partial meniscus tear of the shoulder and knee joints were noted though Grades 2 and 3 partial tears were the most common finding. Finally, it was noted that in the knee joints of the affected patients, the M Me C shaped tear was the major type of tear.(**International Journal of Biomedicine. 2020;10(4):387-391.**)

Key Words: magnetic resonance imaging • shoulder girdle • knee joint injuries • medial meniscal cartilage

Abbreviations

MRI, magnetic resonance imaging; **AGL**, anterior glenoid labral; **MMC**, medial meniscal cartilage; **LCL**, lateral collateral ligament; **MCL**, medial collateral ligament; **ACL**, anterior cruciate ligament; **PCL**, posterior cruciate ligament.

Introduction

The use of MRI has become a routine examination in evaluating and diagnosing musculoskeletal diseases. Nowadays, MRI is used as a preoperative planning method for patients thought to have surgical diseases based on history and physical examination.[(1)]

The knee and shoulder joints are the largest joints of the musculoskeletal system, with complex articulation characterized by the presence of a group of ligament and tendon cartilages, and meniscus structures, that play an important role in stability and mobility. MRI is currently regarded as the reference standard for the diagnosis and evaluation of internal derangements of the knee and shoulder girdle. Due to

its excellent soft-tissue enhancement and multiplanar imaging capabilities, MRI provides significant advantages over other imaging techniques in the evaluation of traumatic injuries of knee and shoulder joints.[2] Ligaments and tendons in both joints provide support and stability to the joints. They allow a normal movement of the joint and any abnormal defect or stress that would render the joint unstable or lead to diseases.[3] When a football player catches his foot in the turf and his whole body weight goes over one joint, this leads to overstress in that joint and this force produces structural damage to the joint structure and ligaments, which is known as a ligament injury.[4]

MRI has high diagnostic performance in the evaluation and assessment of musculoskeletal soft tissue injuries. Nowhere is this more accurate than in the evaluation of the internal structure of joints. MRI is an accurate and cost-effective tool for characterizing a wide spectrum of joint injuries, ranging from ligament injuries to cartilage deficiencies.[5] For radiologists and physicians, evaluation of an injured ligament using MRI requires knowledge of the proper imaging techniques and appropriate protocol, normal and abnormal anatomy, and the clinical significance of detecting abnormalities in the joint.[6]

An optimal MRI technique should include proper patient position, dedicated surface coils, gradient coils, and specific protocols for the suspected diseases. MRI is a powerful method for diagnosing acute and chronic lesions of the stabilizing articular elements and is also useful for evaluating traumatic conditions of the tendons. In normal MRI exams, ligaments and tendons have low signal intensity on MR images, whereas disruption manifests as increased or high signal intensity. Radiologists must be sufficiently aware and possess the expertise to understand the full spectrum of ligament abnormalities and associated MRI findings.[7]

MRI allows optimal diagnosis and evaluates the presence of a tear in the joint, the number of an affected ligament or tendon retraction, and the presence of associated lesions. This information is used to decide the correct surgical plan and surgical approach.[8] Thus, this study aimed to evaluate traumatic injuries of the knee and shoulder joint ligaments by MRI and classify the most common ligament injuries.

Materials and Methods

This study included 50 patients, who were presented in the Radiology Department of Modern Medical Center in Khartoum, in the period from October 2019 to January 2020. The current study adhered to the Declaration of Helsinki and Title 4, US Code of Federal Regulations, Part 46, Protection of Human Subjects.

The sample was divided into two groups: shoulder joint group (Group 1, n=30) with an age range between 16 and 74 years and knee joint group (Group 2, n=20) with an age range between 16 and 77 years.

A waiver of informed consent was conceded as per institutional rules. The inclusion criteria were adult patients with signs and symptoms of ligament tears or musculoskeletal disease in shoulder and knee joints.

Shoulder and knee MRI exams were conducted using General Electric (GE) Signa HD 1.5T MRI scanner (Boston, USA) with an aperture diameter of 60cm. MRI Linux software as an operating system and GE application program. Imaging the shoulder is optimal with a dedicated shoulder coil HD phase array, earplugs to reduce coil noise, and immobilization straps and pads. The patient was in a supine position and careful positioning with the shoulder as close to the center of the magnet as possible. Basic routine sequences are T_1-weighted image – T_1W spin echo (SE), T_1-weighted fast spin echo (FSE), and proton density (PD) fat saturated image (fat-sat) and oriented with axial and coronal orientation in conjunction with coronal, oblique PD FSE fat-sat and sagittal T_2 FSE. To visualize the bicep tendon, labrum, and subscapularis tendon in the shoulder joint, the axial T_2 FSE gradient-recalled echo is a sequence of choice. If the radiologist hesitated about any findings, the short-T_1 inversion recovery in coronal and axial plane was used. The optimal field of view for shoulder and knee MRI examination was 14×16 cm, slice thickness 3.5-4 mm and matrix of 512×512.[7,8]

Imaging the knee is optimal with a coil HD phase array, earplugs, and immobilization pads. The patient lies in a supine position on the couch and the knee is placed within the coil and well immobilized with foam pads. The patient is positioned so that the longitudinal alignment light lies either along the midline of the leg under examination, or displaced from it if the knee has been offset. The knee MRI basic sequences are T_1 SE, T_2 FSE, and PD FSE fat-sat oriented with sagittal and coronal orientation in conjunction with coronal T_2 FSE fat-sat for best visualization of the LCL and MCL. Sagittal T_2 FSE fat-sat is used for the best visualization of the ACL and PCL. Additional information is initiated by applying a special pulse sequence of STIR in the sagittal and coronal plane.[7,8]

In addition, an abnormal high signal intensity in the menisci of the knee and shoulder joint was placed into three broad categories that indicate a partial tear, according to Lotysch et al.[9] A Grade 1 signal is a rounded or amorphous signal in the meniscus that does not disrupt an articular surface. A Grade 2 signal is a linear signal that does not disrupt an articular surface. Grades 1 and 2 signals have been shown to be due to intrasubstance degeneration of the meniscus.[10] A Grade 3 signal is a signal that disrupts an articular surface and indicates a meniscal tear.

Statistical analysis was performed using the standard Statistical Package for the Social Sciences (SPSS Inc., Chicago, IL, USA) version 20 for windows.

Results

In the current study, participants were divided into four age groups (< 25, 26-45, 46-65, and >65 years). The age group most affected with shoulder (46.7%) and knee joint (25%) injuries was 46-65 years (Table 1).

The majority of the patients were males. Their weight ranged from <60 kg up to >81 kg. The weight of 40% of cases ranged from 71-80 kg while the weight of 6.6% was <60 kg. The injuries of the shoulder (40%) and knee joints (55%) were

common in patients with body weight ranging from 71-80 kg and >81 kg, respectively (Table 2).

Table 3 summarizes the frequency and the percentage of the most affected side in the examined joints. It was found that the left shoulder joint was affected in 18(60%) patients while the right knee joint was affected in 11(55%) patients.

Table 1.

Distribution of traumatic injuries of the knee and shoulder joint ligaments in different age groups

Age group (years)	Shoulder joint injuries		Knee joint injuries	
	Frequency	Percentage (%)	Frequency	Percentage (%)
< 25	3	10%	4	20%
26-45	7	23.3%	7	35%
46-65	14	46.7%	5	25%
> 65	6	20%	4	20%

Table 2.

Distribution of traumatic injuries of the knee and shoulder joints, according to body weight

Weight (kg)	Shoulder joint		Knee joint	
	Frequency	Percentage (%)	Frequency	Percentage (%)
<60	2	6.6%	1	5%
61-70	8	26.7%	4	20%
71-80	12	40%	4	20%
> 81	8	26.7%	11	55%

Table 3.

The frequency and the percentage of the most affected side in the examined joints

Affected side	Shoulder joint		Knee joint	
	Frequency	Percentage (%)	Frequency	Percentage (%)
Left	18	60%	9	45%
Right	12	40%	11	55%
Both	0	0	0	0

Different grades of partial meniscus tear of both shoulder and knee joints were noted as Grades 1 and 2 in 8.7% of cases, Grade 2 - 13%, Grades 2 and 3 - 34.8%, and Grade 3 - 30.4% (Table 4).

Finally, there were incidences of 27.3%, 0%, 54.5%, 15.20, 0%, and 3% for ACL, PCL, medial meniscus C-shape (M Me C), lateral meniscus C-shape (L Me C), MCL, and LCL tears in the knee joints of the affected patients (Fig.1)

Table 4.

Complete and different grades of partial meniscus tear of both shoulder and knee joints

Findings	Frequency	Percentage
Complete tear	3	11.5%
Partial tear	23	88.5%
Partial tears, Grades 1 and 2	2	8.7%
Partial tears, Grade 2	3	13%
Partial tears, Grades 2 and 3	8	34.8%
Partial tears, Grade 3	7	30.4%

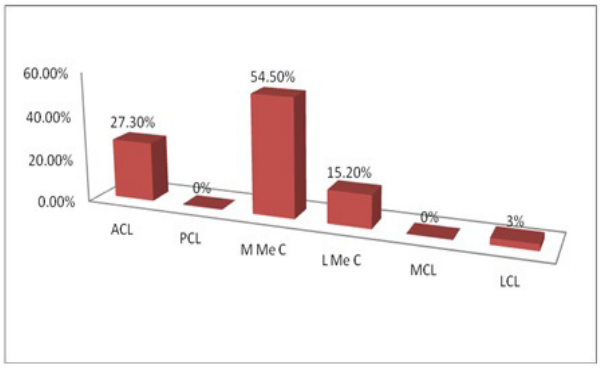

Fig. 1. *Incidence of knee joint ACL, PCL, M Me C shape, L Me C shape, MCL, and LCL tears.*

Discussion

Several imaging modalities, including nuclear medicine, ultrasound, computerized tomography, and MRI, have been used in an attempt to provide a pathophysiological diagnosis in patients with shoulder or knee ligament injuries. The diagnosis of shoulder and knee ligament injuries and diseases has been complicated, so aside from radiological investigations, those modalities guide us to some types of diagnosis and planning. MRI is the method of choice for the routine measurement of shoulder ligament injuries.[4,11,12]

The findings of the current study were compatible with Dinnes et al.,[4] who conclude that in 29 cohort studies there were full-thickness tears. In addition, it was found that the overall MRI sensitivities and specificities were high, and in these studies there were partial-thicknesses detected with MRI in rotator cuff tears, where the supraspinatus tendon was one of this group. Furthermore, two studies suggested that magnetic resonance arthrography might give a more accurate detection of full-thickness rotator cuff tears and shoulder joint injuries.[4,11]

It was found that 3(11.5%) of the patients were presented with a complete menisci tear and 23(88.5%) were presented with a partial meniscus tear. Different grades of partial meniscus tear of both shoulder and knee joints were noted as Grades 1 and 2 in 8.7% of cases, Grade 2 - 13%,

Grades 2 and 3 - 34.8%, and Grade 3 - 30.4%. These findings were compatible with the findings of Mietchen et al.[13] and Englund et al.[14]

Regarding our results shown in Table 1, it should be noted that a Grade 3 signal was most common in both menisci, followed by Grade 2 and Grade 1, which corresponded to studies done by Kanal et al.[7] and Drapé et al.[11] Among menisci, the medial meniscus is most commonly injured (38%), followed by the lateral meniscus (26%). The posterior horn of the medial meniscus was a commonly injured structure among menisci noted in 30 cases out of 64 meniscal tears. The next commonly injured structure is the posterior horn of the lateral meniscus (16.7%), followed by the anterior horn of the lateral meniscus (14.1%), the body of the medial meniscus (12.8%), the body of the lateral meniscus (7.7%), and the anterior horn of the medial meniscus (2.5%). Furthermore, some of the tears that were located in one part extended to other parts of the meniscus. The horizontal tear (12%) was commonest, followed by a complex tear (11%), bucket handle tear (7%), radial tear (3%), and longitudinal tear (2%) in the medial meniscus.[15,16]

In study by Jarvick et al.,[16] the commonest type of tear in the lateral meniscus was a horizontal tear (42.3%), followed by a complex tear (26.9%), longitudinal tear (15.5%), vertical radial tear (11.5%), and oblique tear (3.8%). A similar meniscal injury pattern observed in the present study was comparable to the study of Drapé et al.,[11] in which the most common meniscal injury is the posterior horn of the medial meniscus.

Jarvick et al.[16] and Singh et al.[17] found that among meniscal injuries, medial meniscal tears (46.5%) were more common than lateral meniscus tears (37.2%), and the posterior horn of the medial meniscus (55%) was the most common site of involvement

Finally, findings presented in Table 4 and Fig. 1 of this study could be compared with the findings of Singh et al.[17] and Gupta et al.[18] Those researchers found in two studies (n=100 patients) that a complete MCL tear was noted in one patient, a partial tear in three patients, and a Grade 1 sprain in one patient. Similarly, a partial LCL tear was noted in three patients and a Grade 1 sprain in three patients. It was found that an MCL injury is associated with ACL and medial meniscus injuries. Out of nine MCL injuries, ACL and medial meniscus injuries were noted in six and two patients, respectively. The O'Donoghue triad was noted in one patient. Three lateral meniscal tears were noted in a total of twelve partial LCL tears. LCL tears showed a relationship with lateral meniscus tears.

Regarding the limitations, this study is limited by the heterogeneity of the population because of the randomized selection process, which may influence the exactness of our outcomes and lessen the certainty of our conclusions.

Conclusion

the most affected age group with shoulder and knee joint injuries was 46-65 years. MRI revealed that injuries to the shoulder and knee joints were common in patients with body weight ranging from 71-80 kg and >81 kg, respectively. Different grades of partial meniscus tear of the shoulder and knee joints were noted though Grades 2 and 3 partial tears were the most common finding. Finally, it was noted that in the knee joints of the affected patients, the M Me C shaped tear was the major type of tear.

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Competing Interests

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Radiology

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Measurement of the Diameter of the Common Bile Duct and Pancreatic Duct among Healthy Adult Sudanese Subjects using Magnetic Resonance Cholangiopancreatography

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Abstract

The purpose of our study was to evaluate the common bile duct (CBD) and pancreatic duct (PD) diameter among healthy adult Sudanese subjects using magnetic resonance cholangiopancreatography (MRCP). In addition, this study aimed to determine the effects of age, gender, and body height and weight on the CBD and PD diameters to establish a reference range for these ducts on MRCP, which is very useful in a daily clinical setting where MRCP is commonly performed to evaluate suspected biliary tract disease.

***Methods and Results*:** This study included 80 asymptomatic subjects who underwent MRCP. The widest diameter of the CBD and PD was measured perpendicular to their long axes using the electronic caliper. The age, gender, medical history, body height and body weight were recorded.

Among the 80 subjects, the mean CBD diameter on MRCP was 6.17±0.69 mm (range of 4-8 mm). There was a significant correlation between the CBD diameter and weight (r=0.407, $P<0.001$). The mean PD diameter on MRCP was 3.80±0.50 mm (range of 2-5 mm). There was also a significant correlation between the PD diameter and weight (r=0.407, $P<0.001$). In the cohort of 80 subjects, the mean CBD diameter in females was larger than in males (6.50±0.632 mm and 5.95±0.677 mm, respectively) ($P<0.05$). Also, the mean PD diameter in females was statistically larger than in males (6.03±0.66 mm and 5.58±0.675 mm, respectively) ($P<0.05$). Our results demonstrate no significant correlation between the diameter of CBD and PD and participants' height and age.

***Conclusion*:** The importance of the current study lies in it's being one of the few studies whose intention was to use MRCP to bridge the knowledge gap in the literature about the measurement of the CBD and PD diameter among healthy adult Sudanese subjects. (**International Journal of Biomedicine. 2020;10(4):392-396.**)

Key Words: magnetic resonance cholangiopancreatography • common bile duct • pancreatic duct

Abbreviations

CBD, common bile duct; **CT**, computed tomography; **PD**, pancreatic duct; **MRCP**, magnetic resonance cholangiopancreatography; **BMI**, body mass index.

Introduction

The size of the common bile duct (CBD) is a predictor of biliary obstruction; in this connection an accurate CBD size reference range should be available.[1] A large number of published studies are devoted to the normal size of the CBD; however, an accurate reference range for CBD size remains debatable.[2-5] The availability of an accurate reference range for CBD size would help to distinguish obstructive from non-obstructive causes of jaundice.

Today, MRCP is one of many newer noninvasive tests for assessment of biliary tract abnormality—especially those involving obstruction.[1,5] MRCP provides information on both ductal and parenchymal features of cholangiopancreatography, such as T_1 signal changes, focal or diffuse gland atrophy, and irregular contour of the pancreatic duct (PD).[6]

The diameter of the CBD changes in response to various factors, including age, cholecystectomy, measurement location, respiration, and BMI.[1,3,4,7-9] In 1984, Wu et al.,[10] using real-time ultrasonography, found that the inner diameter of the widest point of the CBD varied from 1 mm to 10 mm, and was found to be age-dependent (r=0.60, P<0.001). Later, other studies supported this observation.[1,3,4,7] However, Horrow et al.[11] obtained controversial results by routine abdominal sonography; they did not find an association between age and size of the extrahepatic bile duct (EHD) in 258 asymptomatic adults. Performing an ultrasound on 1484 normal individuals, Matcuk et al. [2] reported a small increase in EHD diameter with age (+0.02±0.11 mm/y, P<0.001) and female gender (+0.3±1.6 mm, P<0.0001). However, two studies have suggested that gender has no significant effect on the CBD diameter by ultrasound and MRCP.[1,4]

The purpose of our study was to evaluate the CBD and PD diameter among healthy adult Sudanese subjects using MRCP. In addition, this study aimed to determine the effects of age, gender, and body height and weight on the CBD and PD diameters to establish a reference range for these ducts on MRCP, which is very useful in a daily clinical setting where MRCP is commonly performed to evaluate suspected biliary tract disease.

Materials and Methods

The current study adhered to the Declaration of Helsinki and Title 4, US Code of Federal Regulations, Part 46, Protection of Human Subjects. This study was approved by the ethics committee of the College of Medical Radiological Sciences (CMRS), Sudan University of Science and Technology (Khartoum, Sudan). Written informed consent was obtained from each patient.

During the period of October 2019 to July 2020, 80 subjects (40 male and 40 female) aged between 24 and 95 years were enrolled in the study. The age, gender, medical history, list of medications, total serum cholesterol and liver function tests were documented for all subjects. In addition, body height and body weight were also recorded. Subjects with pre-existing hepatobiliary and pancreatic surgery, intra or retroperitoneal tumors, inflammation or hemorrhagic diseases, biliary tract stones, cholecystitis, cirrhosis of the liver, ascites or abnormal liver function tests were excluded from the current study.

MRCP examinations were performed using a 1.5 Tesla Toshiba MRI scanner with 120 mT/m maximum gradient capability and phase array body coil. MRCP examination was performed on patients after an overnight fast of at least 12 hours prior to the examination. After localizing images, the coronal and axial abdominal images were obtained using T_2-weighted pulse sequences. Axial images of the biliary and pancreatic ducts were obtained using a T_2-weighted, fat suppressed pulse sequence. In all subjects, MRCP included a breath-hold, thick-slab, single-shot turbo spin echo (ssTSE BH) sequence images. The parameters of the ssTSE BH sequence were as follows: repetition time (TR) of 3137 msec; effective echo time (TE) of 512 msec; echo train length (ETL) or turbo factor of 128; flip angle of 90°; slice thickness of 30-40mm; field of view (FOV) of 250×250 mm^2; matrix size of 256×205; acquisition time of 8 sec. The same pulse sequence was repeated to acquire 4-6 projections of the pancreatic biliary system from different angles.

Measurements of the CBD and PD diameters were performed independently by an experienced radiological technologist with 20 years of experience with MR techniques. Coronal ssTSE BH images at 4-6 different angular planes were reviewed, and the one with well-demonstrated CBD and BD with the least superimposed artifacts was used for measurement. The widest diameter of the CBD and PD was measured perpendicular to their long axes using the electronic caliper. The applied MRCP imaging technique was in line with the guidelines used by Chen et al.[12]

Statistical analysis was performed using the statistical software package IBM SPSS Statistics for Windows, Version 22.0 (Armonk, NY: IBM Corp.). Continuous variables were presented as mean±standard deviation (SD). Student's unpaired t-test was used to compare two groups for data with normal distribution. Multiple comparisons were performed with one-way ANOVA. 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 the 80 subjects, the mean CBD diameter on MRCP was 6.17±0.69 mm (range of 4-8 mm) (Table 1). There was a significant correlation between the CBD diameter and weight (r=0.407, P<0.001) (Fig. 1). On the other hand, the mean PD diameter on MRCP was 3.80±0.50 mm (range of 2-5 mm). There was also a significant correlation between the PD diameter and weight (r=0.407, P<0.001) (Fig. 2). Furthermore, our results demonstrate no significant correlation between the CBD and PD diameters and participants' height.

In the cohort of 80 subjects, the mean CBD diameter in females was larger than in males (6.4±0.632 mm and 5.95±0.677 mm, respectively), and this difference was statistically significant (P<0.01). Also, the mean PD diameter in females was statistically larger than in males (4.03±0.460

mm and 3.58±0.475 mm, respectively), and this difference was statistically significant (P<0.001) (Table 2).

Table 1.

Descriptive statistic of study variables

Variables	Mean	SD	Minimum	Maximum
Age (years)	57.33	17.098	24	95
Weight (kg)	83.69	9.726	63	105
Height (cm)	167.25	10.06	140	180
CBD diameter (mm)	6. 17	0. 689	4.0	8.0
PD diameter (mm)	3. 80	0.501	2.0	5.0

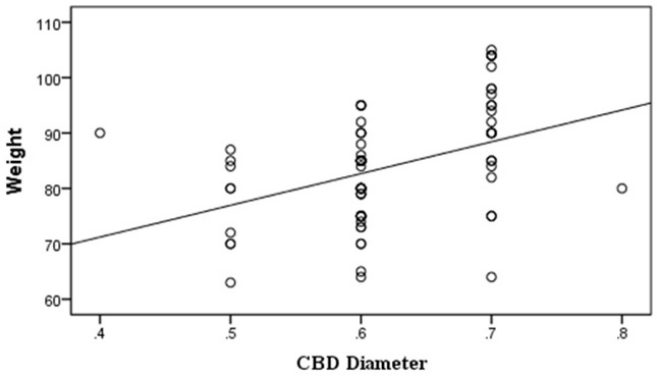

Fig. 1. *Correlation between CBD diameter and weight.*

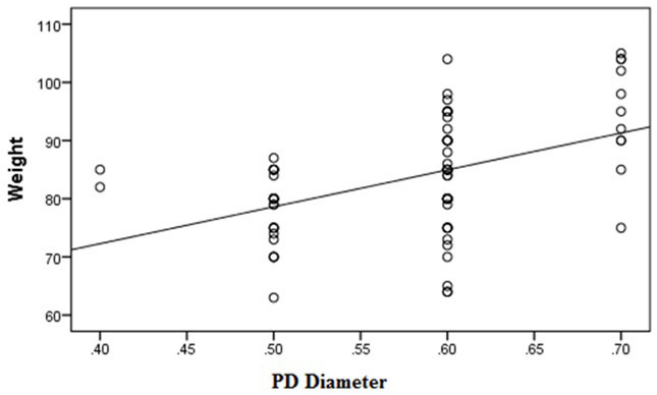

Fig. 2. *Correlation between PD diameter and weight.*

Results demonstrate the mean CBD and PD diameters of subjects in each age group (Table 3). The upper limit of the 95% reference range for the CBD diameter was 6.33 mm, resulting in the reasonable upper limit of 6.5 mm for the asymptomatic subjects. There was no significant correlation between CBD diameters and age. For PD diameter, the upper limit of the 95% reference range was 4.05 mm, resulting in the reasonable upper limit of 4 mm for the asymptomatic subjects. The PD diameter slightly increases until the age of 60 years, then decreases with an increase in age, but results were not statistically significant.

Table 2.

CBD and PD diameters in both genders

Variables	Gender	n	Mean	SD	P-value
CBD diameter (mm)	Female	40	6.4	0.632	0.0029
	Male	40	5.95	0.677	
PD diameter (mm)	Female	40	4.03	0.460	0.0001
	Male	40	3.58	0.475	

Table 3.

Mean CBD and PD diameter in different age group

Age group	n	CBD diameter	PD diameter
20-30	2	6.0±0.01	3.50±0.51
30-40	15	5.87±0.74	3.80±0.58
40-50	19	6.53±0.51	3.89±0.54
50-60	15	6.40±0.63	3.93±0.51
60-70	10	6.0±0.47	3.70±0.48
>70	19	6.0±0.82	3.68±0.47
Total	80	6.17±0.69	3.80±0.50

Discussion

In this study, the mean CBD diameter on MRCP was 6.17±0.69 mm (range of 4-8 mm). Previous studies have shown that the mean CBD diameter was between 3.4 and 7.39 mm, with a range of 1 to 15 mm, and current results were well within the reported range.[1,4,7,13] Our results for the upper limit of the 95% reference range for the CBD diameter (6.5 mm) are comparable to those from ultrasound and CT.[4,7]

A previous study has also suggested that body height and body weight have no significant effect on the CBD diameter.[3] Daradkeh et al.[3] reported that the CBD diameter was correlated with BMI by ultrasound. In our study, subjects' weight had a significant effect on the CBD diameter, but their height did not.

Several reports have considered the important age-dependent variations in the CBD diameter.[1,3,4,7,13] One study revealed a slight increase in duct diameter with advancing age. It has also been shown that the CBD diameter was directly proportional to age after patients were divided into

two groups with 65 years as the cutoff age.[1] Park et al.[13] reported that the CBD diameter by CT in people over age 51 was significantly different from that in subjects under age 50. However, Horrow et al.[11] found no increase in the size of the extrahepatic bile duct with increasing age in an adult population. Our study supports the notion that age has no significant effect on the CBD diameter. Two other studies have reported that gender has no significant effect on the CBD diameter,[1,3] which our findings also support. However, Matcuk et al.[2] reported a small increase in EHD diameter with female gender.

Normal PD diameter in previous publications ranges from 2 mm to 8 mm.[14,15] Our results for the mean PD diameter (range of 2-5 mm) were within the range. The PD diameter slightly increases until the age of 60 years, then decreases as age increases, but results were not statistically significant; nevertheless, they support the notion that increasing age causes atrophy of the pancreatic size. There was also a significant positive correlation between the PD diameter and body weight. These findings are in agreement with the previous studies.[16,17]

This study is limited by the unevenness of the population because of the randomized selection process, which unfortunately might affect the accuracy of our results and, in fact, significantly reduce the power of the conclusions because it makes other age groups have a lower statistical credibility if applied in future studies and may lead to some statistical bias. In addition, our screening questionnaires did not ask the participants for the list of medications that can cause CBD and PD dilatation, such as morphine, calcium antagonists, and nitroglycerine, as mentioned by Chen et al.[12] and Economou et al.[18] Despite the above limitations, the importance of the current study lies in it's being one of the few studies whose intention was to use MRCP to bridge the knowledge gap in the literature about the measurement of the CBD and PD diameter among healthy adult Sudanese subjects.

Conclusion

Our study shows that the average diameter of CBD and PD in the healthy adult Sudanese subjects, measured by MRCP, is 6.17±0.69 mm and 3.80±0.50 mm, respectively. In addition, there was a significant correlation between the diameter of CBD and PD and a participants' weight. The mean diameter of CBD and PD in females was larger than in males, and this difference was statistically significant. Furthermore, our results demonstrate no significant correlation between the diameter of CBD and PD and participants' height and age.

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Competing Interests

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Radiology

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The Pituitary Gland Measurements in Sudanese Females using Magnetic Resonance Imaging

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Abstract

Background: Assessment of the pituitary gland (PG) measurements is essential for the diagnosis of many pathological conditions. For Sudanese adult females, however, there have been no studies and no reference values for PG measurements. Therefore, the aim of this study was to determine the regular dimensions of the PG, using MRI, and to correlate these measurements with age, the shape of sella turcica, puberty age, and parity in Sudanese females.

Methods and Results: This cross-sectional study was done to assess the PG measurement in Sudanese adult females (n=63) aged between 20 years and 60 years who underwent a brain MRI examination between 2015 and 2019. The study was conducted at Yastabshiroon Umodorman Medical Center (Khartoum, Sudan). The MRI brain examination found that the mean length, depth, width, and volume of the PG were 10.57±1.27 mm, 5.56±1.42 mm, 12.18±1.67 mm, and 356.38±100.22 mm^3, respectively. Concerning the shape of the sella turcica, the study revealed that the convex and concave shape were more frequent than others (39.7% and 34.9%, respectively). The depth, width, length, and volume of the gland had changed significantly with pituitary shapes. The PG depth was significantly higher in nulliparous females than multiparous ones.

Conclusion: The PG measurement in adult Sudanese females decreased in the sagittal depth and volume gradually till the age of 50 years then returned to increasing after age 50. Younger females in the age group of 20-30 years had a larger depth and volume of the gland than other age groups. (**International Journal of Biomedicine. 2020;10(4):397-401.**)

Key Words: female • pituitary gland • magnetic resonance imaging • age • parity • puberty age

Introduction

The pituitary gland (PG) lies in the pituitary fossa beneath the body of the sphenoid bone. The size of the sphenoid sinus and sella turcica, including the depth and shape of the PG, usually have significant variations in different individuals.(1) MRI is an accurate diagnostic imaging method for assessing the PG size and shape. Changes are noted in the pituitary signal's size, shape, and intensity, reflecting changes in the gland's hormonal function. There are several pituitary disorders, such as physiological hypertrophy, inflammatory changes, and empty sella turcica. The shape and size of the normal PG often change during one's lifespan and are affected by age and gender.(2,3)

Previous studies have found that there is a wide variation of pituitary size associated with age and gender. The variation in PG volume depends on hormonal status. Thus, due to hormonal activity younger adults show a larger pituitary during puberty and, in females, during pregnancy.[4-8]

MRI of PG in females is essential to assess females' pituitary size and volume. It has been reported that PG is affected by age, parity, puberty age, and hormone-related factors.

Materials and Methods

This cross-sectional study was done to assess the PG measurement in Sudanese adult females (n=63) aged between 20 years and 60 years who underwent a brain MRI examination between 2015 and 2019. The study was conducted at Yastabshiroon Umodorman Medical Center (Khartoum, Sudan). Written informed consent was obtained from each patient.

The data were collected from 63 Sudanese adult females between 20 and 60 years of age, classified into 4 age groups (mean age of 35.52±11.11 years) in puberty and menopausal categories, and puberty age ranged between 10 years and 19 years. The sample was selected randomly. Inclusion criteria were Sudanese women of indicated age with no medical or pathological condition that may affect PG shape and measurement. The exclusion criteria were pituitary abnormalities or previous intracranial surgery. Any woman with a disturbance of pituitary hormones or schizophrenia was excluded from the study.

MRI technique

MRI of the brain was performed using a General Electric Healthcare (GE SIGNA EXCITE) machine with a 0.2T permanent magnet (open magnet). A standard head coil was used for acquiring the images. The sagittal and axial views were displayed using the midline plane of T1-weighted image spine-echo, matrix size was 512×512, FOV was 24×18 (repetition time/echo time of 450/10.5 ms, 378/8.6 ms), and slice thickness of 6.3 mm. Foam pads and straps were used for immobilization. The patient lay supine on the examination table with the head placed carefully in the head coil. The head was adjusted with the interpupillary line parallel to the table. The longitudinal alignment light lies were adjusted in the midline and horizontal alignment to pass through the nasion. Three axial sequences, T1-weighted image, T2-weighted image, and fluid-attenuated inversion recovery (FLAIR) were utilized. T1 sagittal image and T2 coronal image with additional sequences were used to assess the pituitary measurements. PG was measured in thin, sagittal, axial sections. The measurements were done in the Dicom imager program. The sagittal depth of PG was measured as the pituitary's vertical distance, defined by a line connecting two maximum top-bottom points in the T1-weighted sagittal section (Fig. 1A). The maximum anteroposterior diameter called (sagittal length) was measured as the longitudinal distance, defined by the line connecting two corners of the PG longitudinally (Fig. 1B) T1-weighted sagittal section. The axial width was measured by the maximum two-point distances from the right to left borders of the PG in the axial section (Fig. 1C).

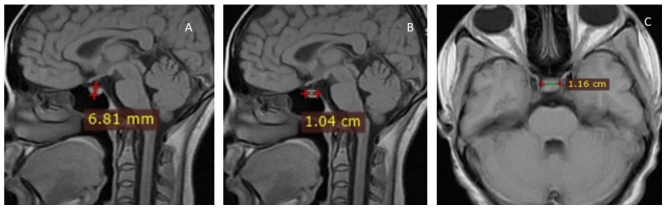

Fig. 1. *MRI images show pituitary measurements taken with spin echo sequence, TR 600, TE 14. A & B - T1-weighted sagittal images; C - T1-weighted axial image taken with TR 350 and TE 7.8.*

The PG volume was calculated using an ellipsoid object's formula:

Di Chiro and Nesion formula (Vol = length × depth × width × 0.5).

The shape of the PG in the sagittal view according to the upper surface was determined as: "Convex" (round shape) (Fig. 2A); "Concave" (Fig. 2B); "Flat" (Fig. 2C); Partial empty (PE) - the gland high of 3-4 mm (Fig. 2D); Empty sella turcica - the gland height ≤2 mm (Fig. 2E).

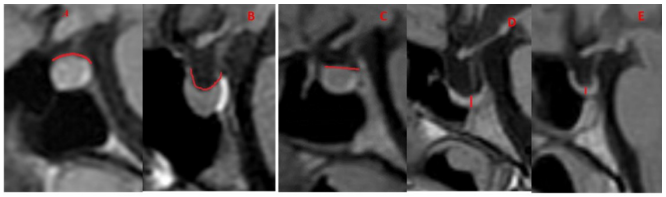

Fig.2. *Different shapes of the sella turcica. A: Convex shape, B: Concave shape, C: Flat shape, D: Partial empty, E: Empty shape*

Statistical analysis was performed using IBM SPSS Statistics 23. Continuous variables were presented as mean±standard deviation (SD). Multiple comparisons were performed with one-way ANOVA The linear regression model and coefficient of determination (R^2) were calculated. 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

The MRI brain examination found that the mean length, depth, width, and volume of the PG were 10.57±1.27 mm, 5.56±1.42 mm, 12.18±1.67 mm, and 356.38±100.2 $2mm^3$, respectively (Table 1).

Concerning the shape of the sella turcica, the study revealed that the convex and concave shape were more frequent than others (39.7% and 34.9%, respectively) (Table (2).

The depth, width, length, and volume of the gland had changed significantly with pituitary shapes. It was observed that the flat shape yields the highest volume, followed by the convex, then concave, while the partially empty and empty

sella turcica yield a lower volume. The sagittal depth was higher in the convex shape (6.75±0.75 mm), and axial width was higher in the partially empty sella turcica (13.02±1.59 mm). The sagittal length and volume were higher in flat-shaped sella turcica (11.76±0.7 mm and 419.89±31.72 mm^3, respectively) (Table 3).

Table1.

Descriptive statistics for PG measurement

Variables	Minimum	Maximum	Mean±SD
Age, years	20	60	35.52±11.11
Puberty age, years	10	19	14.16±1.54
Sagittal length, mm	6.1	13.5	10.57±1.27
Sagittal depth, mm	2.0	8.3	5.56±1.42
Axial width, mm	8.4	15.5	12.18±1.67
Volume, mm^3	98.0	563.1	356.38±100.22

Table 2.

The frequency of shapes of the sella turcica

Shape	Frequency	Percent
Convex	25	39.7
Concave	22	34.9
Flat	9	14.3
Partial empty	5	7.9
Empty	2	3.2
Total	63	100.0

Table 3.

Association of PG measurements with pituitary shape, age groups, and puberty age

Variables	Sagittal length, mm	Sagittal depth, mm	Axial width, mm	Volume, mm^3
Pituitary shapes				
Convex	10.25 ±1.22	6.75±0.75	11.54±1.72	398.63±84.31
Concave	10.46±1.26	5.08±0.63	12.69±1.54	337.55±71.32
Flat	11.76±0.71	5.68±0.67	12.69±1.30	419.89±31.72
Partial empty	10.83±1.60	3.02±0.43	13.02±1.59	216.70±67.96
Empty	9.85±0.21	1.96±0.00	10.25±0.35	98.90±1.28
P-value	0.030	0.000	0.030	0.000
Age groups, years				
20-30	10.23±1.82	6.14±1.25	11.79±1.49	371.387±101.25
31-40	10.90±1.47	5.17±1.10	12.52±1.87	350.54±97.96
41-50	10.29±0.98	5.04±1.77	12.47±1.59	326.47±118.52
51-60	11.50±0.67	5.32±1.45	12.33±2.01	367.92±71.40
P-value	<0.05	>0.05	>0.05	>0.05
Puberty age, years				
10-13	10.91 ±0.87	5.45±1.41	12.25±1.72	366.83±105.56
14-16	10.24±10.72	5.80±1.45	12.02±1.69	351.98±100.51
17-19	10.72±1.32	4.79±1.06	12.81±1.46	324.29±71.07
P-value	> 0.05	> 0.05	> 0.05	> 0.05

There was a significant difference in sagittal length in different age groups ($P<0.05$); it increased gradually till the age of 40, then decreased in length in the age group of 41-50 years. It increased significantly in the 51-60 group. There was no significant difference in sagittal depth, axial width, and volume measurement in different age groups. The mean depth in the age group of 20-30 years was 6.14±1.25mm, while in the age group of 41-50 it was 5.04±1.77 mm, and then it returned to increase to 5.32±1.45mm in the age group of 51-60 years. The volume was 371.387±101.25 mm^3 in the age group of 20-30 years, then it decreased to 326.47±118.52 mm^3 in the age group of 41-50 years and returned to increase again after 50 years to 367.92±71.40 mm^3 (Table 3).

The PG measurement has no significant association with the puberty age. Generally, the volume and depth were higher at the early puberty age than in the late one. The volume was 366.83±105.56 mm^3 and 324.29±71.07 mm^3 in puberty age of 10-13 years and 17-19 years, respectively. The depth was 5.45±1.41 mm and 4.79±1.06 mm in puberty age of 10-13 years and 17-19 years, respectively (Table 3).

The PG sagittal depth was significantly different among parity groups ($P<0.05$). The depth was significantly higher in nulliparous females than multiparous ones (Table 4). The other pituitary measurements (sagittal length, axial width, and volume) didn't vary significantly with parity; in general the volume also was greater in nulliparous than in multiparous women (Table 4).

Table 4.

PG measurement and parity groups

Parity	Sagittal length, mm	Sagittal depth, mm	Axial width, mm	Volume, mm^3
Nulliparous	10.47±1.43	6.18±1.20	11.93±1.66	379.32±80.66
1-3	10.18±1.36	4.61±1.59	12.18±1.68	294.81±132.60
4-6	10.93±1.12	5.36±1.38	12.45±1.78	362.28±101.91
7-10	10.55±1.03	5.20±1.35	12.35±1.68	341.53±101.22
P-value	>0.05	<0.05	>0.05	

The study found no significant correlation of the PG measurements with age, puberty age, and parity (Table 5).

Table 5.

Correlation of the PG measurements with age, puberty age, and parity

Variables		Age	Puberty age	Parity
Sagittal length, mm	Pearson Correlation	0.221	-0.172	0.045
	Sig. (2-tailed)	0.082	0.178	0.728
Sagittal depth. mm	Pearson Correlation	-0.235	-0.006	-0.235
	Sig. (2-tailed)	0.064	0.963	0.064
Axial width, mm	Pearson Correlation	0.099	-0.041	0.145
	Sig. (2-tailed)	0.440	0.752	0.257
Volume, mm^3	Pearson Correlation	-0.089	-0.139	-0.097
	Sig. (2-tailed)	0.490	0.277	0.451

Table 6 summarizes the association of the sella turcica shape with age groups. The convex shape was prominent in the age group of 20-30 years, while the concave shape was frequent in the age group of 31-40 years. This finding indicates that the sella turcica shape was significantly different among age groups (*P*=0.028).

Table 6.

Cross tabulation between age groups and shape of the sella turcica

Shape	Age group				Total
	20-30	31-40	41-50	51-60	
Convex	16	4	3	2	25
Concave	7	9	5	1	22
Flat	2	1	2	4	9
Partial empty	0	2	2	1	5
Empty	1	0	1	0	2
Total	26	16	13	8	63
P=0.028*					

There was an inverse relationship between females' age and pituitary depth and volume (Fig.3-5). From this linear correlation, the study predicts the depth and volume of the gland by identifying the female's age, as follows:

PG depth= -0.0304×age+6.6496 (R^2=0.0563)

PG volume= -0.08×age+384.81 (R^2=0.0079)

The following equations indicate an inverse relationship between the gland's volume and puberty age (Fig.5):

PG depth= -9.0162×puberty age+484.05 (R^2=0.0194)

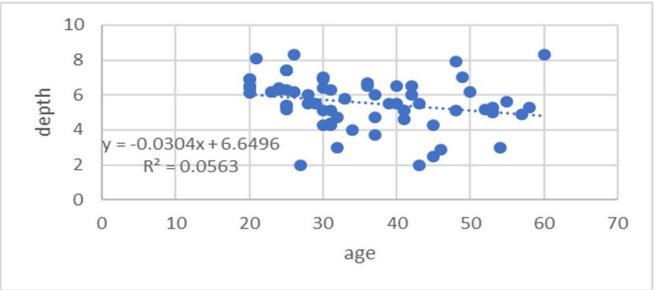

Fig.3. *Linear relationship between PG depth and age.*

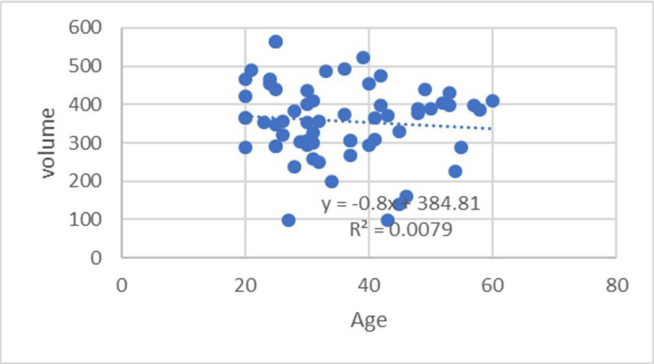

Fig. 4. *Linear relationship between PG volume and age*

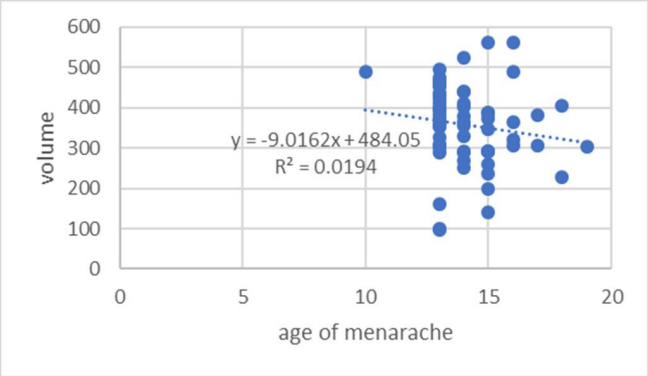

Fig. 5. *Linear relationship between volume and puberty age*

Discussion

The study evaluated the shape, mean volume, depth, width, and length of the normal PG in females, related to age, puberty age, and parity using MRI. In this study, females' pituitary height was higher than in a study performed by A. Tsunoda,[4] who reported a mean value of 5.35±1.2 mm. The PG height was less in this study than in a study performed by Singh et al.[10] (5.80±1.32 mm). Ibinaiye et al.[9] reported that the mean PG volume for females in North Nigeria was 328.1±129.2 mm^3. Our findings for the PG agree with data of Singh et al.[10] (354.98±130.60 mm^3).

The current study found that the convex and concave shapes were more common than other shapes. In contrast, Yadav et al.[2] found that the most common shape was flat. Singh et al.[10] found that convex in females was mostly seen in the age group of 10–29 years. In our study, convex shapes were more frequent in the age group of 20-30 years.

The current study clarified that the PG yielded the most considerable depth and volume in females of 20-30 years. The depth and volume then declined till the age of 50 years, and then returned to increasing. Yadav et al.[2] found that in the age group of 21-30 years, the mean PG volume was 440±180 mm^3, in the 31-40 group - 440±111 mm^3, in 41-50 group - 420±116 mm^3, and in females over age 50 - 420±174mm^3. Our findings agree with data of Ibinaiye et al.,[9] who reported that the PG depth and volume increased in pubertal subjects, then decreased steadily with increasing age, with a second peak noted only for pituitary height in the sixth decade.

Our study found that PG depth yielded the highest value in the age group of 20-30 years, then declined till the age of 50 years. After age 50, the depth returned to increasing. This finding is consistent with Yadav et al.[2] and Tsunoda et al.[4], who reported the same results. Tsunoda et al.[4] found that the PG height reached a peak value in the age group of 20-29 years, then returned to increasing again in the age group of 50-59. Doraiswamy,[8] on the other hand, found that the depth decreased until the age of 59 and increased from 60-69 years. In contrast, many studies found that the PG reached the highest value of depth in the third decade of life,[2,4,8] while other studies found that the PG yields the largest peak at the age of 10-19 years.[3,11] Previous studies attributed the changes in pituitary measurements to changes in hormone levels,

which might be the cause of such changes in the pituitary morphology. The elevation in PG height during puberty can be related to the increased production of a luteinizing hormone during this time of growth. Also, the greater PG height was observed in young patients.[2,8,11,12]

Previous studies have reported that PG depth increases in elderly subjects, which is considered compensatory hypertrophy after a significant reduction in a gonadal steroid feedback effect. The pituitary size is greater in adolescence due to physiology hypertrophy in females, which occurs as a result of changes in hormones associated with menstruation; the reduced PG size between the second and sixth decades of life may reflect neuroendocrinology of aging and physiologic pituitary atrophy.[2,6, 8,11,12]

The study found that there was a significant difference in the sagittal length of the PG measurement in different age groups. It increased gradually until the age of 40 years, then decreased at age 41-50 years and increased significantly at age 51-60 years. A significant difference in sagittal depth measurement was found in different parity groups and was more significant in nulliparous than multiparous women. In our study, the nulliparous females had a larger PG than other groups of parity. This finding is consistent with Daghighi et al.,[13] who stated that the gland's volume is greatest in nulliparous women.

Concerning the PG measurement in females with PG of different shapes, a significant difference was found between measurement in glands of different shapes as the flat shape yielded the highest volume followed by convex then concave, while the partially empty and empty sella turcica yield a lower volume.

We found no significant correlation between gland measurements, age, puberty age and parity. In general, as female age, puberty age, and parity increased, sagittal depth and volume of the PG decreased. There was an insignificant inverse correlation between the depth, the volume of PG with age, puberty, and parity. In contrast, Daghighi et al.[13] found that gravidity and parity had a significant negative impact on PG volume (P<o.o1). The insignificant relationship in our study may be due to the small sample size.

Conclusion

Thus, the mean measurements (length, depth, width, and size) of PG in females changed significantly with the pituitary shapes. The PG measurement in adult Sudanese females decreased in the sagittal depth and volume gradually till the age of 50 years then returned to increasing after age 50. Younger females in the age group of 20-30 years had a larger depth and volume of the gland than other age groups. The pituitary volume increased in females with early onset of puberty age, and in nulliparous females. The convex-shaped gland was more prevalent in younger individuals of 20-30 years. Further studies with a larger sample size with a hormonal profile should be performed for more accurate results.

Competing Interests

The authors declare that they have no competing interests.

Acknowledgments

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ORIGINAL ARTICLE

Radiology

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Evaluation of Liver in Type 2 Diabetes Mellitus Using Unenhanced Computed Tomography

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Abstract

Background: Chronic liver disease occurs due to different etiologies. Most diabetic patients are unaware that the effective control of hyperglycemia might reduce complications and mortality rates. Fatty liver disease is considered a risk factor of hepatic cirrhosis and cancers.

Methods and Results: We conducted a case-control study to assess the impact of type 2 diabetes mellitus (T2DM) on the liver using a CT scan. A total of 100 patients with T2DM and 96 non-diabetic patients as a control group were selected using a convenient sampling method. There was a significant difference in liver attenuation in diabetic and control groups. The CT attenuation values of the liver, pancreas, and spleen were significantly lower in patients with T2DM than in non-diabetics (P<0.001). There was a significant negative correlation between the duration of T2DM and CT attenuation of the liver, pancreas, and spleen (P<0.01).

Conclusion: The CT attenuation of the liver was significantly lower in T2DM than in the non-diabetic patients, and liver attenuation decreased as the duration of T2DM increased. (**International Journal of Biomedicine. 2020;10(4):402-406.**)

Key Words: diabetes mellitus • nonalcoholic fatty liver disease • liver attenuation • unenhanced computed tomography

Abbreviations

BMI, body mass index; **CT**, computed tomography; **DM**, diabetes mellitus; **HS**, hepatic steatosis; **HU**, Hounsfield Unit; **HbA1c**, glycosylated hemoglobin; **L/S**, liver-to-spleen ratio; **LP**, liver-to-pancreas ratio; **LLL**, left liver lobe; **NAFLD**, nonalcoholic fatty liver disease; **PACS**, picture archiving and communication system; **RLL**, right liver lobe; **T2DM**, type 2 DM.

Introduction

Type 2 diabetes mellitus (T2DM) is a metabolic disorder that can affect most parts of the body, including the liver. Insulin resistance mainly causes hyperglycemia, which affects the metabolism of carbohydrates, lipids, and proteins leading to nonalcoholic fatty liver disease (NAFLD). The fatty liver may progress to steatohepatitis, hepatic cirrhosis, fibrosis, and hepatocellular carcinomas. Several studies have found that DM is associated with several hepatic abnormalities, such as NAFLD, cirrhosis, fibrosis, cancers, acute liver disease, unusual elevated hepatic enzymes, and hepatitis. Furthermore, increased fat accumulation in the hepatocytes may affect insulin

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resistance and end in severe metabolic dysfunction. A fatty liver and hyperglycemia can damage the hepatocytes and lead to severe morbidity and mortality among T2DM patients.[1]

CT is considered a reliable method for assessing fatty liver. CT is capable of calculating the liver-to-spleen (L/S) ratio, which is defined as the ratio of the intensities of the liver parenchyma and splenic texture. It is considered an accurate imaging modality for quantifying HS with 90% accuracy.[2,3]

The estimated CT attenuation values of liver parenchyma on an unenhanced CT scan show a strong correlation with the degree of HS seen on histopathologic examination. The measured CT attenuation value of liver parenchyma on an unenhanced CT scan has been reported to show a strong association with the severity of HS shown on histopathological analysis.[4]

The attenuation of a healthy normal liver on unenhanced CT is denser than the spleen and the hepatic vasculature, and it ranges from 50HU to 65HU.[5,6] Non-enhanced CT is established as an accurate imaging method for diagnosing moderate to severe fatty liver than it is for mild conditions of steatosis. HS is diagnosed when liver attenuation is less than spleen attenuation or when liver attenuation less than 48HU.[6] The degree of HS can be diagnosed when liver attenuation is less than 40-42HU, and L/S attenuation ratio is less than 0.8, or L/S ratio difference is equal or less than 10HU.[7,8]

CT is a useful modality for measuring fat in the liver for patients at risk of metabolic syndromes, such as T2DM. Therefore, this study aimed to assess the liver in T2DM using unenhanced CT, focusing on assessing CT attenuation of the diabetic liver, compared to the non-diabetic individuals.

Materials and Methods

The study was conducted in the Medical Imaging Department – CT scan department in Jeddah Hospitals. This study was approved by the ethics committee of the Sudan University of Sciences and Technology. Written informed consent was obtained from each patient.

The cases were retrospectively studied from the reviewed database of all patients who had undergone abdominal CT examinations from March 2018 to March 2020. The exclusion criteria were fibrosis, alcohol consumption, and history of hepatic surgery. A total of 196 patients were selected using a convenient sampling method. Among them, 100 subjects had T2DM regarded as cases, and 96 were non-diabetics as controls.

Unenhanced CT scans of the liver were performed for all participants included in the study. The abdominal CT examinations were done using TOSHPA CT Machine, at 120kVp, 50mA-100mA, 5-mm slice thickness (1mm for Axial, 1mm for Coronal). Collimation was 0.5×80, pitch of 0.8. Every patient was examined in supine positioning, typically feet first, scanning from above the diaphragm (top of the liver) to the level of the iliac crests. The patients were asked to hold their breath at the end of inspiration.

Attenuation measurements

We delineated five regions of interest: LLL (segment III), RLL (segment V), RLL (segment VI), middle of the spleen, and the body of the pancreas on the CT scans of each patient. The attenuation measurements were obtained for each ROI, which include a larger area of the liver and spleen. Regions excluded were of non-uniform parenchymal attenuation, including apparent hepatic vessels. The 2D axial and coronal images were used to measure the size of the liver and spleen. All the images were interpreted by a single observer with 15 years of experience in CT scan and PACS.

Statistical analysis was performed using statistical software package SPSS version 23.0 (SPSS Inc, Chicago, IL). For descriptive analysis, results are presented as mean ± standard deviation (SD). Means of 2 continuous normally distributed variables were compared by independent samples Student's t test. Group comparisons with respect to categorical variables are performed using chi-square 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

The study included 100 patients with T2DM (case group) (54% males and 46% females) and 96 non-diabetic patients (control group) (42.7% males and 57.3% females). The age of T2DM patients ranged from 31 to 85 years (mean age of 58.91±11.1 years); mean BMI was 30.74±7.25 kg/m^2. The results showed that in T2DM, the mean level of HbA1c was 7.4%±1.9%. The mean age of non-diabetic patients was 45.96 years; mean BMI - 27.59±5.9 kg/m^2 (Table 1).

Table 1.

Characteristics of the study groups

Variables	T2DM patients	Non-diabetic patients	*P*-value
Male	54(54%)	41(42.7%)	0.668
Female	46(46%)	55(57.3)	
Age, years	58.91)	45.96	0.001
BMI, kg/m^2	30.74±7.25	27.59±5.90	0.001
HbA1c,%	7.47±1.91	--	
Duration, ,years	14.62±7.19	--	

Concerning T2DM, the study clarified that the attenuation of the liver in the different segments was as follows: 44.62±9.93 HU in segment III of LLL, 43.46±9.77HU in segment V of RLL, and 41.29±9.63 HU in segment VI of RLL. The CT attenuation of the spleen was 51.88±9.13 HU, and the pancreas was 34.58±8.74 HU. The liver indexes were 203.08 ±22.92 mm in axial one, 117.15±4.62 mm in axial two, and 165.85±28.75 mm in coronal one. The spleen indexes were 96.32±17.50 mm in axial 1, 42.76±9.71mm in axial 2, and 87.84±20.72 mm in coronal section (Table 2).

Table 3 summarizes the correlation between attenuation (HU) of liver and spleen and duration of DM. The study

shows a moderate, negative, significant correlation between the attenuation and DM duration. In contrast, a strong, negative significant correlation was found between pancreas HU and DM duration (r=-574, P<0.01). Thus, CT attenuation decreased as the duration of T2DM increased. HbA1c values also had a moderate, negative, significant correlation with the liver, spleen, and pancreas attenuation (P<0.01).

Table 2.

Means measurement for age, BMI, duration, liver HU and indexes in T2DM patients

Variables	Mean±SD
LLL, HU (segment III)	44.62±9.93
RLL, HU (segment V)	43.46±9.77
RLL, HU (segment VI)	41.29±9.63
Spleen, HU (middle)	51.88±9.13
Pancreas, HU (body)	34.58±8.74
Liver Index (Axial 1)/mm	203.08 ±22.92
Liver Index (Axial 2)/mm	117.15 ±14.62
Liver Index (Coronal)/mm	165.85±28.75
Spleen Index (Axial 1)/mm	96.32±17.50
Spleen Index (Axial 2)/mm	42.76±9.71
Spleen Index (Coronal)/mm	87.84±20.72
L/S ratio	0.83
L/P ratio	1.2

Table 3.

Correlation between the duration of DM and HU of liver, spleen, and pancreas.

Correlations		Liver (upper)	Liver (middle)	Liver (lower)	Spleen (middle)	Pancreas (middle)
Duration	Pearson Correlation	-0.375*	-0.430*	-0.415*	-0.411*	-0.574*
	Sig. (2-tailed)	0.000	0.000	0.000	0.000	<0.001
HbA1c	Pearson Correlation	-0.402*	-0.484*	-0.505*	-0.459*	-0.453*
	Sig. (2-tailed)	0.000	0.000	0.000	0.000	<0.001

**- Correlation is significant at the 0.01 level (2-tailed).*

A comparison of the HU attenuation of liver and spleen in DM and non-DM patients clarified that the liver's attenuation in diabetic patients is significantly lower than in non-diabetic patients. The CT attenuation of LLL (segment III) in T2DM patients was significantly lower than in non-diabetics (56.2±10.69 HU vs. 44.62±9.93 HU, P<0.01). In T2DM patients and non-diabetic patients, the CT attenuation of RLL (segment V) was 43.46±9.77HU and 56.02±10.65HU, respectively, (P<0.01) and 44.62±9.93HU and 56.2±10.69HU, respectively, for segment VI (P<0.01) (Table 4).

Table 4.

Independent sample t-test to compare means of HU and indexes for liver, spleen, and pancreas in diabetic and non- diabetic patients

Variables	Mean±SD		P-value
	T2DM patients (n=100)	Non-diabetic patients (n=96)	
LLL, HU (segment III)	44.62±9.93	56.20±10.96	< 0.01
RLL, HU (segment V)	43.46±9.77	56.02±10.65	
RLL, HU (segment VI)	41.29±9.63	55.84±11.08	
Spleen, HU (middle)	51.88±9.13	47.15±8.84	
Pancreas, HU (body)	34.58±8.74	45.91±9.44	
Liver Index (Axial 1)	203.08 ±22.92	201.95±20.46	> 0.05
Liver Index (Axial 2)	117.15 ±14.62	114.35±20.03	
Liver Index (Coronal)	165.85±28.75	159.44±29.55	
Spleen Index (Axial 1)	96.32±17.50	95.2±15.74	
Spleen Index (Axial 2)	42.76±9.71	42.98±8.55	
Spleen Index (Coronal)	87.84±20.72	89.19±16.99	

The spleen attenuation values in T2DM patients were significantly higher than in non-diabetics (51.88±9.13 HU vs. 47.15±8.84 HU, P<0.001). On the other hand, the pancreatic attenuation values in T2DM were lower than those in non-diabetic patients (34.58±8.74 HU vs. 45.91±9.44 HU, P<0.01) (Table 4).

The study found no significant difference in liver and spleen indexes in T2DM patients versus non-diabetic patients. The liver indexes, in general, were slightly higher in T2DM- than in non-diabetic patients. In T2DM patients, the indexes were 203.08±22.92 mm, 117.15±14.62 mm, and 165.85±28.75 mm in axial 1, axial 2, and coronal section, respectively; in non-diabetic patients the indexes were 201.95±20.46 mm, 114.35±20.03 mm, and 159.44±29.55 mm in axial 1, axial 2, and coronal section, respectively.

Discussion

There are many imaging techniques to evaluate the liver. Several previous studies recommended unenhanced CT because of measurements of fat in the useful liver modality for patients at risk of metabolic syndrome, such as diabetes mellitus. CT allows quantitative assessment of the liver attenuation in HU.

In this study, we used CT since it is useful in diagnosing the presence of liver fat and assessing its severity safely.

The present study revealed that fatty infiltration of the liver was significantly correlated with T2DM. The prevalence of NAFLD is highest in populations with metabolic conditions such as obesity and T2DM. Specifically, T2DM and NAFLD are closely related. A study of patients with T2DM found that fatty liver is significantly associated with DM characteristics, even at younger ages.[9]

The present study found that the CT attenuation values of the liver were significantly decreased in T2DM more than in non-diabetic participants, in agreement with previous studies.[10,11] This decrease in attenuation value of the liver is attributed to the fact that the attenuation value of fat, usually about -100 HU, is much lower than that of soft tissue, which ranges from 30 HU to 40 HU. Therefore, the attenuation value of liver parenchyma decreases as HS develops and progresses. This finding indicates that CT assessment of liver parenchyma on an unenhanced CT scan is accurate since a strong correlation was reported between CT and histopathological analysis regarding the diagnosis of hepatic steatosis.[3]

It was found that the L/S ratio was 0.83 in T2DM patients. This finding is in agreement with previous studies, which reported approximately similar results.[12,13] On an unenhanced CT scan, the normal liver parenchyma is slightly higher than that of the spleen. As fatty hepatic infiltration progresses, the attenuation value of liver parenchyma decreases, and consequently attenuation of the liver to spleen decreases.[2,3] Therefore, the L/S ratio is a significant indicator of a CT assessment of NAFLD.

In this study, we found that a negative correlation existed between the duration of DM and attenuation values of the pancreas, liver, and spleen. As the duration of the DM increased, the attenuation of the liver, pancreas, and spleen decreased significantly (P<0.001). We also found that the most affected organ was the pancreas, which showed a strong, significant negative correlation.

Pancreatic fat density decreased accordingly as the duration of the disease increased.[14] Similarly, Lim et al.[15] stated that T2DM patients had excessive pancreatic fat content, compared to normoglycemic subjects. Ahbab et al.[16] reported that DM correlated with a decrease in the mean HU values of the pancreas (P=0.002). These decreased attenuation values were attributed to the fact that pancreatic fat content increased in T2DM, resulting in increased fat content in the pancreatic tissue. The negative correlations suggested that the values of these factors increase as the degree of pancreatic fatty infiltration increases. In general, these findings indicate that the duration of T2DM is a strong influencing factor affecting the CT attenuation of the pancreas, liver, and spleen.

Conclusion

The study determined that an unenhanced CT scan evaluation of the liver in T2DM is necessary to determine the degree of fatty infiltration. The CT attenuation values of the liver, pancreas, and spleen were lower in diabetic patients than non-diabetic ones. The attenuation values of the liver, spleen, and pancreas were significantly decreased as the duration of DM increased.

Competing Interests

The authors declare that they have no competing interests.

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Sustained Attention and Types of Dynamics of Cardiovascular Reactivity during a Short-Term, Human Whole-Body Exposure to Cold Air

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Abstract

Background: Despite global warming and the improvement of personal protective equipment against unfavorable climatic factors, cold remains an important environmental challenge for humans. The aim of the work was to reveal the peculiarities of the dynamics of cardiovascular parameters in humans with short-term, whole-body exposure to cold air, depending on the parameters of voluntary attention.

Methods and Results: The study involved 28 healthy male volunteers aged between18 and 21 years, born and living in Arkhangelsk. Testing to assess sustained attention parameters using the Toulouse-Pierón Attention Test with the measurement of the index of accuracy (C, units) (the ability to voluntary concentration) and processing speed (V, units), had been previously performed. Subsequent stages of the study included recording the studied parameters before (Stage 1), during 10-minute exposure to the cold air (Stage 2), and 5 minutes after cold exposure (Stage 3). The registration of indicators in Stages 1 and 3 was carried out indoors at an air temperature of +20 °C. The registration of indicators in Stage 2 was carried out in a cold chamber at -20 °C. Determining the body temperature in the ear canal (T_{ear},°C) and on the skin of the dorsum of the right hand (T_{skin},°C) was performed using a B.Well WF-1000 medical electronic infrared thermometer. Parameters of blood pressure (SBP and DBP) and heart rate variability (HRV) were evaluated.

As a result of cluster analysis, 2 groups were identified: Group 1 (n=14) and Group 2 (n=14). In Group 2, the index V was significantly lower than in Group 1 (P=0.02). In Group I, T_{skin} in Stage 1 was significantly higher than in Group 2 (P=0.03). In Stage 2, Tskin decreased in both groups, but lower T_{skin} values (P=0.001) were recorded in Group 2 than in Group I. In Stage 2, there was a statistically significant increase in SBP in Group 2 (P=0.01). In Group 1, initial SDNN and all HRV spectral indices were significantly higher than in Group 2. In Stage 2, there was a significant increase in SDNN in both groups. However, in Group 2, we found a statistically significant increase in VLF in Stage 2 (P=0.01), while in Group I this indicator remained unchanged. In Stage 3, HRV parameters in Group 1 recovered to baseline values, while in Group 2, HRV parameters remained elevated relative to baseline values.

Conclusion: Individuals with high processing speed and preserved attention span have higher vagal activity and skin temperature. When such individuals are exposed to cold, they experience a moderate increase in BP and baroreflex response. In persons with a reduced speed of information processing but with sufficient accuracy of attention, there is a more pronounced mobilization of regulation resources on the part of the cardiovascular, neurovegetative systems to maintain the core temperature of the body.(**International Journal of Biomedicine. 2020;10(4):407-411.**)

Key Words: sustained attention • air cooling • cardiovascular reactivity

Abbreviations

BP, blood pressure; **CIG**, cardiointervalogram; **DBP**, diastolic BP; **HR**, heart rate; **HRV**, heart rate variability; **SI**, stress index; **SBP**, systolic BP; **TP**, total power.

Introduction

Despite global warming and the improvement of personal protective equipment against unfavorable climatic factors, cold remains an important environmental challenge for humans. An active study and development of the resource potential of the Arctic will be determined by a person's ability to adapt while maintaining his health and high mental

performance.[1,2] The impact of cold leads to a decrease in the functional reserves of the human body and to a decrease in mental performance.[3] During the migration of the population to the North and during the long-term adaptation of migrants to the conditions of high latitudes, the human cardiovascular system is one of the first to react.[4] HRV is formed under the control of the central nervous system and is associated with human mental health and such cognitive functions as memory and voluntary attention. Several studies have shown that adolescents living in the North have a lower ability to switch attention than adolescents living in more southern regions. In both adults and adolescents living in the North, sensorimotor reactions may be slower.[5] The question remains, to what extent are the psychophysiological properties of a person related to the strategies of adaptive changes in regulatory systems when exposed to cold?

The aim of the work was to reveal the peculiarities of the dynamics of cardiovascular parameters in humans with short-term, whole-body exposure to cold air, depending on the parameters of voluntary attention.

Materials and Methods

The study involved 28 healthy male volunteers aged between18 and 21 years, born and living in Arkhangelsk. The study was conducted in accordance with ethical principles of the WMA Declaration of Helsinki (1964, ed. 2013) and approved by the Ethics Committee of FECIAR UrB RAS (Protocol №2 of 03.28.2018). Written informed consent was obtained from all participants.

Volunteers had no signs of deficiency or excessive body weight (BMI of 18.5-25 kg/m^2), or fever (*axillary temperature* ≤37°C).

The studies were carried out in the winter (January-February) in the morning in an office with a comfortable temperature regime. The exclusion criteria of the study were cardiovascular and neurological diseases, and increased sensitivity to cold (including Raynaud's syndrome).

Testing to assess sustained attention parameters using the Toulouse-Pierón Attention Test with the measurement of the index of accuracy (C, units) (the ability to voluntary concentration) and processing speed (V, units), had been previously performed. Index C was calculated as the ratio of correctly processed characters to the total number of characters processed; the index V was the number of characters processed per minute.[6]

Subsequent stages of the study included recording the studied parameters before (Stage 1), during 10-minute exposure to the cold air (Stage 2), and 5 minutes after cold exposure (Stage 3). Volunteers dressed in underwear, cotton trousers, winter boots and a cotton robe were examined in a sitting position at resting-state. The registration of indicators in Stages 1 and 3 was carried out indoors at an air temperature of +20°C. The registration of indicators in Stage 2 was carried out in a cold chamber at -20°C. Determining the body temperature in the ear canal (T_{ear}, °C) and on the skin of the dorsum of the right hand (T_{skin}, °C) was performed using a B.Well WF-1000 medical electronic infrared thermometer (Switzerland) before entering the chamber (Stage 1), on the 10th minute of being in the chamber (Stage 2) and the 5th minute after leaving the chamber (Stage 3).

BP (SBP and DBP) was determined using an A&D medical device (Japan) before entering the chamber, immediately after leaving the chamber and in the 5th minute after leaving the chamber.

For the possibility of CIG recording under cold room conditions, we used a one-lead electrocardiogram channel of the Neuron-Spectrum-SM device, placed in a tank with thermal insulation. Subsequently, CIGs of the last 5 minutes in the cold chamber (Stage 2) and Stages 1 and 3 were processed using the Varicard instrument software (Ramena, Russia), and the HRV parameters were calculated. The following HRV parameters were evaluated: HR – heart rate, bpm; SDNN (msec) – the standard deviation of the normal-to-normal RR intervals; SI (units) –Stress Index, calculated by the formula: SI=Amo50/2×VAR×Mo, where Mo (msec) is the cardiointerval value dividing the CIG series in half; VAR – variation range between the minimum and maximum values in the CIG series, Amo50,% –amplitude of mode – number of R-R intervals; TP (msec^2) – Total Power of the HRV spectrum, HF (msec^2) – High Frequency Power of the HRV spectrum (0.15 to 0.40 Hz); LF (msec^2) – Low-Frequency Power of the HRV spectrum (0.04-0.15 Hz); VLF (msec^2) – Very Low Frequency power of the HRV spectrum (0.0033–0.04 Hz).

Statistical analysis was performed using the statistical software «Statistica» (v. 13.0, StatSoft, USA). The normality of distribution of continuous variables was tested by Shapiro-Wilk's W test. Median (Me) and interquartile range (IQR; 25th to 75th percentiles) were calculated. The Mann-Whitney test was used to compare differences between two independent groups. The Friedman test was used to test for differences between 3 dependent samples, followed by post-hoc analysis with Wilcoxon signed rank test (P<0.017). A clustering analysis using the k-means algorithm was performed.

Results

As a result of cluster analysis, 2 groups were identified: Group 1 (n=14) and Group 2 (n=14). In Group 2, the index V was significantly lower than in Group 1 (P=0.02) (Table 1).

Table 1.

Parameters of sustained attention in humans before whole-body exposure to cold air, Me (P_{25}; P_{75})

Parameter	Group 1 (n=14)	Group 2 (n=14)	*P*-value
C, units	0.97 (0.94;0.97)	0.97 (0.96;0.98)	0.190
V, units	58.9 (52.2;65.6)	50.7 (41.9;55.9)	0.020

In Group I, T_{skin} in Stage 1 was significantly higher than in Group 2 (P=0.03) (Table 2). In Stage 2, T_{skin} decreased in both groups, but lower T_{skin} values (P=0.001) were recorded in

young people in Group 2 than in Group I. T_{ear} also decreased in both groups and remained decreased in Stage 3, as did T_{skin}. In Stage 2, there was a tendency to increasing SBP in Group 1 (P=0.02) and a statistically significant increase in SBP in Group 2 (P=0.01). In Stage 2, DBP in both groups increased significantly (P=0.001). In Stage 3, DBP was similar to baseline values.

In Stage 1, HR in both groups was within the normal range, but in Group 2, its value was significantly higher than in Group 1. In Stage 3, HR was significantly lower, compared to the baseline value in both groups (P<0.001).

In Group 1, initial SDNN and all HRV spectral indices were significantly higher than in Group 2. In Stage 2, there was a significant increase in SDNN and a decrease in SI in both groups. However, in Group 2, we found a statistically significant increase in VLF in Stage 2 (P=0.01), while in Group I this indicator remained unchanged. In Stage 3, HRV parameters in Group 1 recovered to baseline values, while in Group 2, HRV parameters remained elevated relative to baseline values.

Discussion

Participants in Group 1 with a relatively high speed of information processing had a more pronounced baseline vagal activity and higher baseline T_{skin} values than those in Group 2. SI values in Group 2 subjects reflected the trend of basic sympathicotonia.

A number of researchers believe that the activity of the divisions of the autonomic nervous system, reflected

Table 2.

Body temperature and cardiovascular parameters in humans during whole-body exposure to cold air (Me (P_{25}; P_{75})

Parameters	Group 1 (n=14)			*P* Friedman	Group 2 (n=14)			*P* Friedman
	Stage I	Stage 2	Stage 3		Stage I	Stage 2	Stage 3	
	1	2	3		1	2	3	
T_{skin}, ^{0}C	34.5 (29.0;35.7)	20.1 (19.1;21.1) ##(1-2)	24.1 (23.9;25.7) ##(1-3)	<0.001	27.7 (26.2;33.0) *	16.9 (19.1;18.9) **##(1-2)	22.1 (21.1;24.0) ##(1-3)	<0.001
T_{ear}, ^{0}C	36.3 (36.1;36.3)	34.1 (33.6;34.8) ##(1-2)	35.2 (34.7;35.6) ##(1-3)	0.001	36.3 (36.1;36.4)	34.5 (33.6;34.7) ##(1-2)	35.0 (34.8;35.7) ##(1-3)	0.001
SBP, mmHg	127 (121;130)	132 (123;136)	123.5 (117;130) ##(2-3)	0.003	121.5 (112;133)	134 (120;140) ##(1-2)	127 (112;132) ##(2-3)	0.006
DBP, mmHg	82 (80;87)	87 (86;97) ##(1-2)	82 (78;90) ##(2-3)	0.001	82.5 (71;91)	97 (81;104) ##(1-2)	89.5 (78;91) #(2-3)	0.001
HR, bpm	70.9 (62.3;76.2)	70.2 (64.3;75.4)	65.1 (61.1;68.6) ##(1-3)	<0.001	78.9 (71.4;88.7) *	76.5 (71.5;80.1)	70.1 (64.6;76.8) ##(1-3)	0.003
SDNN, msec	72.5 (44.7;84.9)	92.1 (82.8;131.4) ##(1-2)	76.5 (60.0;106.8)	0.001	41.9 (36.3;49.3) **	78.5 (55.9;82.2) ##(1-2)	66.3 (51.5;106.3) ##(1-3)	<0.001
SI, unit	49.2 (30.5;100.5)	30.9 (17.6;53.6) ##(1-2)	38.9 (18.2;59.6)	<0.001	132.2 (94.6;176.8) **	46.6 (36.9;85.4) ##(1-2)	54.7 (25.3;102.6) ##(1-3)	<0.001
TP×1000, msec^2	3.77 (2.81;6.15)	7.16 (4.36;13.95) ##(1-2)	5.68 (3.95;10.13)	0.005	1.78 (1.24;2.31) **	4.69 (2.94;6.21) ##(1-2)	3.76 (2.63;7.13) ##(1-3)	<0.001
HF×1000, msec^2	1.02 (0.72;1.69)	2.64 (1.14;3.04) ##(1-2)	1.12 (0.74;1.62)	0.002	0.43 (0.24;0.58) **	1.12 (0.83;1.65) ##(1-2)	0.77 (0.49;1.21) ##(1-3)	<0.001
LF×1000, msec^2	1.20 (0.96;1.92)	2.22 (1.38;4.66) ##(1-2)	1.97 (1.08;3.45)	0.01	0.72 (0.34;0.98) **	1.52 (0.84;3.20) ##(1-2)	1.13 (0.69;2.99) ##(1-3)	0.004
VLF ×1000, msec^2	0.95 (0.56;1.62)	0.95 (0.66;2.51)	1.16 (0.78;1.53)	0.807	0.30 (0.20;0.40) **	0.57 (0.32;0.74) ##(1-2)	1.07 (0.51;1.53) ##(1-3)	0.031

**P<0.05, ** P<0.01 - between groups in same stage (Mann-Whitney test); ## P<0.01 - between stages in each group (Wilcoxon test).*

in the parameters of the variability of cardiac activity, is associated with the regulation of attention processes.[7] The frontal thalamic system, which includes the prefrontal cortex and mediodorsal nucleus of the thalamus,[8] is the main neural regulatory system for voluntary attention.There is also a functional relationship between HRV parameters and the activity of the prefrontal cortex,[9] as well as between vagus tone and attention control processes.[7] Higher HRV is associated with better cognitive functions such as memory and attention.[10] G. Park showed that people with lower HRV needed more time to solve problems with distractions; at the same time, participants with a higher HRV showed a more effective control of selective attention.[10,11] A. Hansen et al.[12] showed that male sailors with high HRV had a faster response to a stimulus, more correct answers, and fewer errors than the group with low HRV. It is believed that sympathetic influences on HR function are relatively slow (on the order of seconds) compared to the effects of the vagus nerve (on the order of milliseconds). Thus, in a person with a relative deficit in vagal modulation on the HR, the ability to track environmental challenges and the reactivity of the nervous system is reduced.[7,9]

When exposed to cold, afferent sensory information from peripheral skin thermoreceptors is integrated in the hypothalamus, subsequently contributing to peripheral vasoconstriction, especially in the skin area through the activation of adrenergic nerve fibers. Despite an identical decline rate T_{ear} in both groups, which is closest to the core of the body, it is obvious that individuals with a lower concentration of attention (Group 2) experienced a more pronounced decrease in T_{skin}, which reflects a more pronounced effect of peripheral vasoconstriction to limit heat transfer and maintain core temperature.

The pronounced adrenergic activation in response to cold also caused a significant reaction of the stroke volume in the great vessels and a pronounced increase in not only DBP but also SBP in persons of Group 2. As a response to a sharp increase in BP, we observed a decrease in baroreflex modulation of HR, and an increase in total HRV and LF band of the HRV spectrum in both groups. However, persons with a lower speed of voluntary attention had a more pronounced response of VLF band of the HRV spectrum to the effect of cold, which indicates the activation of the central ergotropic mechanisms of regulation;[13] that is, in persons with a lower speed of information processing and with a tendency to basic sympathicotonia, the response to cooling will be more pronounced with the involvement of the central mechanisms of the autonomic nervous system regulation of HR. It is assumed that this type of reaction is aimed at long-term resistance of the body to the damaging effects of cold.

In conclusion, individuals with high processing speed and preserved attention span have higher vagal activity and skin temperature. When such individuals are exposed to cold, they experience a moderate increase in BP and baroreflex response. Such an adaptive strategy can be in demand either with short-term exposure to cold or in individuals with high adaptive capabilities of thermogenesis with a minimal shift in hemodynamic parameters.

In persons with a reduced speed of information processing but with sufficient accuracy of attention, there is a more pronounced mobilization of regulation resources on the part of the cardiovascular, neurovegetative systems to maintain the core temperature of the body. Such an adaptive strategy can be biologically expedient for the human body under conditions of long-term exposure to cold, for which large energy resources of the body can be involved. However, with more pronounced sympathicotonia and persistence of high BP after exposure to cold, the risk for cold hypertension and tissue damage increases.

Competing Interests

The authors declare that they have no competing interests.

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Using Ki-67 Mitotic Activity Markers as a Predictor of the Progression of Adhesions in the Abdominal Cavity

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Abstract

Background: Ki-67 is a nuclear protein expressed in all proliferating cells of vertebrates during mitotic cycle phases S, G1, G2, and M, except for G0. Studying this marker is widely used to diagnose the proliferative activity of tumors. However, studying Ki-67 in non-neoplastic diseases attracts much less attention among the researchers. The aim of this study was to assess the possibility of using staining for Ki-67 to identify the proliferative potential of fibroblasts during the formation of adhesions in the abdominal cavity (AC).

Methods and Results: Experiments were carried out on male Wistar rats. The adhesion process in AC was simulated in the control group (n=25), and in the experimental group (n=25) with the administration of Seroguard®. Animals were sacrificed on Days 1–30, and the severity of the adhesive process in AC was assessed. Histological sections were prepared and stained for Ki-67. It was found that the animals of the control group had increased severity of the adhesive process in AC during the observation. Maximum increase in severity was registered on Day 30 – 12[9-13] points in the control group and 4[4-4] points in the experimental group (P=0.0079). High proliferative activity of fibroblasts in the control group was detected on Days 3, 7, 14 and 30, which may indicate an active division of fibroblasts and the formation of adhesions in the damaged area. In the experimental group, single Ki-67 positive cells were noted during the entire observation period, which may point to a reduced potential for the formation of adhesions.

Conclusion: Our study showed the prospects of using Ki-67 staining to determine the severity of the developing adhesive process in AC, and also revealed one of the possible mechanisms that inhibit the formation of the adhesive process when using Seroguard® – a decrease in the mitotic activity of fibroblasts in the area of peritoneal injury. (**International Journal of Biomedicine. 2020;10(4):412-415.**)

Key Words: adhesive process • mitotic activity • Ki-67 • p38 MAPK

Abbreviations

AC, abdominal cavity; **mRNA**, messenger ribonucleic acid; **p38 MAPK**, p38 mitogen-activated protein kinases.

Introduction

Ki-67 is a nuclear protein expressed in all proliferating cells of vertebrates during mitotic cycle phases S, G1, G2, and M, except for G0.[(1)] Ki-67 was first identified as an antigen in the nuclei of Hodgkin's lymphoma cells.[(2)] In cultured cells, Ki-67 levels are the highest in the G2 phase and in mitosis.[(3)]

Previously, it was thought that Ki-67 is constantly regulated and used by proteasomes in the cell nucleus. However, a pathway was recently discovered for eliminating extranuclear Ki-67, through which it is transported to the Golgi apparatus.[(4)] It was shown that in normal cells Ki-67 is a late marker of entry into the cell cycle; Ki-67 mRNA fluctuated with maximum levels in G2, while Ki-67 protein levels increased throughout the cell cycle, peaking in mitosis. After exit from the cell cycle, Ki-67 expression remains at a low level, but is not detected in uncycling differentiated cells or senescent cells.[(5)]

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Studying this marker is widely used to diagnose the proliferative activity of tumors, in particular, breast tumors,[6,7] pulmonary tumors,[8] prostate tumors,[9] renal tumors,[10] gastric tumors,[11] and glioblastomas.[12,13] In breast tumors, Ki-67 study is included in the diagnostic standard of morphological examination. However, studying Ki-67 in non-neoplastic diseases attracts much less attention among the researchers. P. Betz et al.,[14] when studying wounds of human skin, drew attention to the fact that fibroblasts in the wound area showed an increased number of Ki-67 positive cells, which could be detected for the first time in skin lesions with a duration of 1.5 days. Positive results were consistently detected from 6 days to 1.5 months after the wounding. Only in the scar tissue of the oldest (7 months) wound, positively stained fibroblasts were not detected.

In several researches, studying Ki-67 expression is used to assess the activity of fibrosis formation and the effectiveness of treatment, in particular, in idiopathic pulmonary fibrosis,[15] peripheral arterial restenosis,[16] rheumatoid arthritis,[17] systemic sclerosis,[18] leiomyoma of the body of the uterus and adenomyosis.[19]

El-Zammar et al.[20] registered low proliferative activity of fibroblasts in keloid scars using the Ki-67 marker. The same observation was made in hereditary gingival fibromatosis [21] and lipofibromatosis.[22]

The Ki-67 marker is also used to assess the proliferative activity of cells in experimental studies.[23-25] It has been shown that Ki-67 is not expressed in mature adhesions in AC.[26] At the same time, Ki-67 stained cells were not detected at all in peritoneal tissue samples from patients without adhesions.[27]

The aim of this study was to assess the possibility of using staining for Ki-67 to identify the proliferative potential of fibroblasts during the formation of adhesions in the abdominal cavity (AC).

Materials and Methods

The experiments were carried out in 50 male Wistar rats. The adhesion process in AC was simulated by our own method, which is described in detail in patent RU 2467401.[28]

The studies were carried out in two groups: Group 1 (control group, n=25) – modeling the adhesive process; Group 2 (experimental group, n=25) – modeling the adhesive process with administration of Seroguard® (conjugate the 4-[4-(4-fluorophenyl)-2-(4-methylsulfylphenyl)-1H-imidazole-5-pyridine with poly-1-vinylimidazole, JSC Pharmasyntez), 3 ml during the completion of the operation.[29]

The animals were sacrificed on Days 1, 3, 7, 14, and 30. The severity of the adhesive process in AC was assessed using our own scale.[30] The material from the adhesion formation zone was fixed using FineFix solution (Milestone, Italy); preparation and paraffin embedding were carried out according to the classical method.[31]

Sections were prepared and stained by the immunofluorescence method.[32-34] Antibodies to Ki-67 rabbit polyclonal (Abbiotec, Cat. N 250733, Lot 09092202) in a dilution of 1:300 were used as primary antibodies; Alexa fluor 568 goat anti-rabbit IgG (H+L) (Invitrogen, Cat. N A-11036 Lot 757102) in a dilution of 1:300 were used as secondary antibodies. Nuclei were stained with Hoechst 33342 (Molecular Probes).

The percentage of positively stained cells on the histological specimen was calculated. During statistical processing, the median, 25% and 75% quartiles were determined.

The experiments were performed in accordance with the norms for the humane treatment of animals and approved by the Ethics Committee of the Irkutsk Scientific Center of Surgery and Traumatology.

Results

At the first stage of the study, we carried out a point assessment of the severity of the adhesive process in AC. As a result, we found that in Group 1 there was an increase in the severity of the adhesive process during the observation, and maximum increase in the severity was registered on Day 30 – 12[9-13] points out of 16 possible points according to score scale (Figure 1). In Group 2, there was no significant increase in the severity of the adhesive process; by Day 30 it was estimated at 4[4-4] points (Figure 2). Differences in the severity of the adhesive process between the groups are significant on Days 7, 14 and 30: on Day 7 in Group 1, the score was 9[8–12] points, in Group 2 – 4[4-6] points (P=0.0159); on Day 14 – 9[6-9] and 3[3-4] points, respectively (P=0.0159). The differences between the groups increased to Day 30 – up to 12[9-13] and 4[4-4] points (P=0.0079).

To study the mitotic activity of cells in the zone of adhesion formation, we used staining for Ki-67 antigen, a marker of cell proliferation. One day after modeling the adhesive process in Group 1, only single Ki-67 positive cells were noted; in Group 2 such cells were not detected at the same timepoint.

The study carried out on Day 3, corresponding to the start of the fibroblastic phase of inflammation in animals of Group 1, showed the maximum number of Ki-67 positive cells – 18[5-28]%, which indicates a high proliferative potential of cells in the damaged zone. Morphologically, these cells correspond to fibroblasts of young connective tissue (Figure 3). High proliferative activity persisted in Group 1 on Days 7 (7[5-7]%), 14(10[8-15]%), and 30(12 [6-18.5]%), which may indicate the continuation of active division of fibroblasts and adhesion formation in the damaged area (Figure 1).

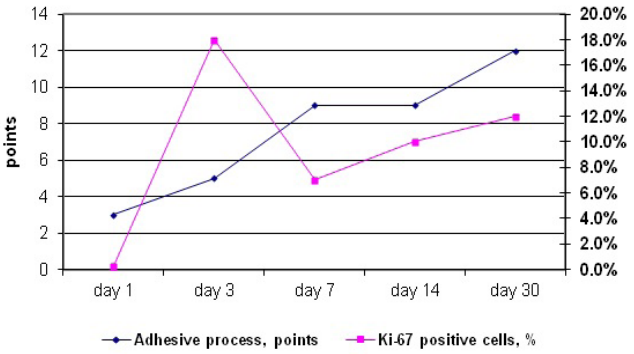

Fig. 1. *Adhesion and Ki67 expression in the control group.*

In Group 2, during the entire observation period, single Ki-67 positive cells were noted (on Day 3 – 0[0-0], Day 7 – 0[0-1], Day 14 – 0[0-1], and Day 30 – 1[0-2.5]), which may be evidence of a reduced proliferative activity of fibroblasts in the peritoneal injury zone and reflect a reduced potential for adhesion formation (Figures 2,3).

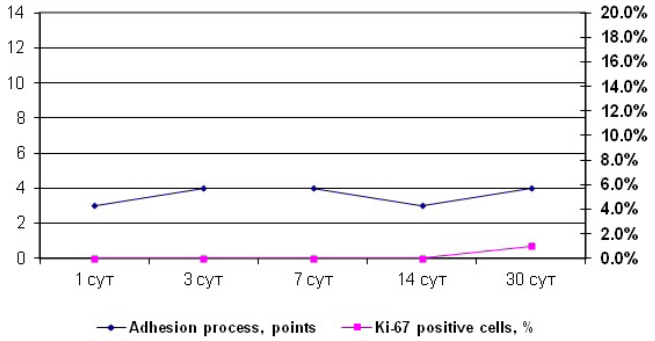

Fig. 2. *Adhesion and Ki67 expression in the experimental group.*

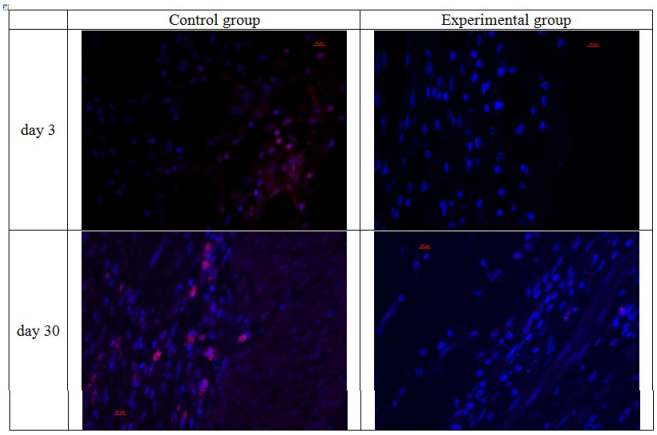

Fig. 3. *Staining for Ki-67, immunofluorescence, primary antibodies to Ki-67 (Abbiotec), 1:300, secondary antibodies Alexa Fluor 568, Hoechst 33342.*

Comparison of the dynamics of the formation of the adhesive process in the studied groups is shown in Figures 1, 2. Differences between the groups are significant on Days 3, 7, 14, and 30 of observation.

Discussion

We have clearly demonstrated that positive staining of fibroblast nuclei for Ki-67 in the area of peritoneal injury is a predictor for the severity of adhesion in AC. This observation is confirmed by the high level of Ki-67 positive cells in the control group, where the formation of the adhesive process is observed in dynamics – 12 points, which corresponds to Grade 3 according to the scale.[35] At the same time, a low index for cells with a Ki-67 positive phenotype in the experimental group causes the formation of a Grade 1 adhesion process, estimated at 4 points. This observation of low activity of mitotic processes in fibroblasts in the repair zone when using p38 MAPK blockers (experimental group) is combined in this group with an increase in apoptosis processes,[36] which together determines a low potential for adhesion formation.

Conclusion

Our study showed the prospects of using Ki-67 staining to determine the severity of the developing adhesive process in AC, and also revealed one of the possible mechanisms that inhibit the formation of the adhesive process when using Seroguard® – a decrease in the mitotic activity of fibroblasts in the area of peritoneal injury.

Competing Interests

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

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The Level of Certain Interleukins and Tissue Factor in Conditions of Intestinal Ischemia and Reperfusion in Rats

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Abstract

Background: The aim of this study was to estimate the blood levels of interleukins (ILs) and tissue factor (TF) in rat blood when modeling acute mesenteric ischemia (AMI) in various stages and during reperfusion.

Methods and Results: The study was performed on white non-linear male rats, weighing 200±25g. AMI was simulated by ligating the cranial mesenteric artery at the mesentery root. Then, at certain time intervals, a relaparotomy was performed, blood was collected, and the animals were subjected to reperfusion at different time intervals.

During the experiment, the animals were divided into 3 groups depending on the time of ischemia (3 hours [n=10], 6 hours [n=10], and 8 hours [n=10]) followed by reperfusion after the indicated time of ischemia.

The level of ILs (IL-6, IL-8, and IL-10) and TF in rat blood serum was determined by EIA. We found that AMI in rats is accompanied by pronounced changes in the immune system and the development of a systemic inflammatory reaction syndrome, which is aggravated by reperfusion. At the same time, the restoration of blood flow after 3-hour ischemia was characterized by an increase in the concentration of IL-6, IL-8, and IL-10; after 6-hour ischemia – by an increase in the IL-6, IL-8 content and TF, and stabilization of the IL-10 concentration. Reperfusion after 8-hour ischemia was accompanied by an increase in the IL-6 concentration, a decrease in the levels of IL-8, IL-10, and TF, which can lead to the progression of necrotic changes in the intestine.

Conclusion: The detected changes can serve as laboratory markers that characterize the course and stage of acute mesenteric ischemia. (**International Journal of Biomedicine. 2020;10(4):416-420.**)

Key Words: acute mesenteric ischemia • rats • immunity • lymphocytes

Abbreviations

AMI, acute mesenteric ischemia; **EIA**, enzyme immunoassay; **IL**, interleukin; **TF**, tissue factor.

Introduction

Despite the current progress in understanding the pathogenetic mechanisms of acute mesenteric ischemia (AMI), AMI remains a serious diagnostic problem, which contributes to maintaining a high level of mortality in patients.[1] Early diagnosis and rapid surgical intervention are necessary for adequate recovery of mesenteric blood flow, prevention of intestinal necrosis, and improvement of the clinical outcome of the disease.[2] Unsatisfactory results of this pathology's diagnostics are mainly due to the lack of a typical clinical picture, the low sensitivity of ultrasound and laparoscopic types of research, low availability of accurate diagnostic methods, and the lack of specific laboratory markers of the disease.[3]

Cytokines play an important role in the development and course of various diseases, including those of the digestive organs.[4] With the help of these specific proteins, a

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wide interaction is realized at the subcellular, cellular, organ, and system levels, forming a complex protective reaction aimed at neutralizing pathogens, destroying them, eliminating them from the body, and preserving the body's structural and functional integrity.[5] In the body, cytokines interact closely with each other, forming a universal biological communication network that triggers and regulates a cascade of inflammatory, immune, and metabolic processes.[6]

An important factor involved in the formation of atherothrombosis is the tissue factor (TF), which is also closely related to the immune-inflammatory process, endothelial dysfunction, angiogenesis, and cell migration processes, all of which play a significant role not only in the development of cardiovascular diseases but also in the occurrence of a number of inflammatory and oncological processes.[7] Clarification of AMI pathogenesis based on the analysis of immunological indicators will help to improve the quality of diagnostics and improve pathogenetic therapy, which will improve the results of care for patients with this pathology.

The aim of this study was to estimate the blood levels of interleukin and TF in rat blood when modeling AMI in various stages and during reperfusion.

Materials and Methods

The study was performed on white non-linear male rats, weighing 200±25g. The experiment was conducted on the basis of a certified vivarium of the Chita State Medical Academy. AMI was simulated by ligating the cranial mesenteric artery at the mesentery root.[8] Termination of the blood flow through the artery was controlled visually: paling of the artery distal to the ligation site, desolation of the marginal vessels of the small intestine, and the cessation of their pulsation. After the operation, the animal was placed in a separate box, where it remained until the end of the experiment. Then, at certain time intervals, a relaparotomy was performed, blood was collected, and the animals were subjected to reperfusion at different time intervals. The resumption of blood flow through the artery was monitored visually: filling of the artery distal to the ligation site, filling of the marginal vessels of the small intestine, and the appearance of their pulsation. The wound was sutured; the animal was placed in a separate box. A day after reperfusion, under general anesthesia, a relaparotomy was performed, as well as intestinal viability assessment, blood sampling, and histological material sampling; then the animals were withdrawn from the experiment.

During the experiment, the animals were divided into 3 groups depending on the time of ischemia (3 hours [n=10], 6 hours [n=10], and 8 hours [n=10]) followed by reperfusion after the indicated time of ischemia.

The level of ILs and TF in rat blood serum was determined by EIA using test kits Cusabio Biotech Co., Ltd (China) for IL-6, Ray Biotech, Inc. (Germany) for IL-8 and IL-10, and Rat Tissue Factor (TF) ELISA (USA). All stages of the reaction were carried out on ST-3 shaker-incubators (Latvia) under thermostatically controlled conditions. Reaction tracking, calibration graphs, and concentration determination were performed on the ANTHOS 2010 vertical scanning photometer (Austria) using LabTech software.

The operations and all manipulations with animals were performed using general anesthesia, and euthanasia - by overdosing on drugs for anesthesia, taking into account the provisions regulated by ethical standards. Work on the animals was done in compliance with the principles of the Helsinki Declaration on the humane treatment of animals, stated in normative documents of the European community(86/609/EU), Manual on Experimental (Preclinical) Study of New Pharmacological Substances,[9] and "Good laboratory practice" (MHRF Order No. 708н dated 23.08.2010).

The study was approved by the Ethics Committee of the Chita State Medical Academy.

Statistical analysis was performed using the Statistica 6.1 software package (Stat-Soft Inc., USA). The normality of distribution of continuous variables was tested by the Kolmogorov-Smirnov test with the Lilliefors correction and Shapiro-Wilk test. For descriptive analysis, results are presented as mean±standard deviation (SD). The Wilcoxon criterion was used to compare the differences between the paired samples. A probability value of $P \leq 0.05$ was considered statistically significant.

Results and Discussion

When studying the dynamics of cytokines in the blood of experimental animals on the AMI model, the indicators during ischemia were compared with the initial data, and the parameters after reperfusion were compared with similar indicators during ischemia.

After 3 hours of ischemia, we found an increase in the serum content of IL-6 and IL-8 by 10.1 times ($P<0.01$) and 4.1 times ($P<0.05$), respectively, relative to the initial data (Fig.1).

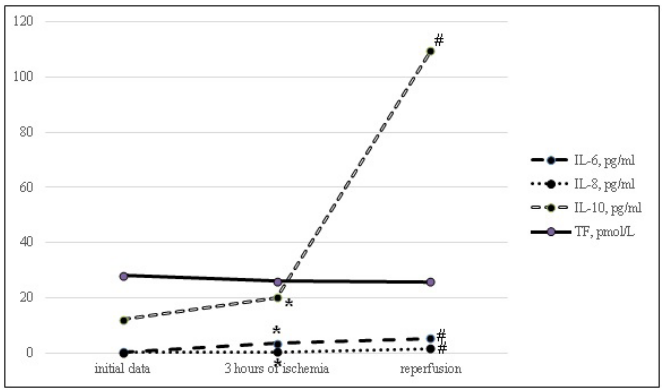

Fig. 1. *Changes in the levels of ILs and TF after 3-hour ischemia and reperfusion (* - statistically significant differences with initial data, # - statistically significant differences between 3-hour ischemia and reperfusion).*

Recovery of mesenteric blood circulation (reperfusion), in comparison with data after 3-hour ischemia, caused an even greater increase in the IL-6 concentration by 1.53 times ($P<0.05$) and IL-8 by 3.47 times ($P<0.05$). The level of

IL-10 after 3 hours of ischemia increased by 1.67 relative to the initial level (P<0.05), while reperfusion caused a sharp increase in the cytokine concentration – by 5.5 times, compared to data with ischemia (P<0.01). No statistically significant results were obtained for the TF indicator (P>0.05).

After 6 hours of ischemia, the serum concentration of IL-6 increased by 17.35 times (P<0.01), in comparison with the initial data, and reperfusion contributed to the growth of this indicator by 1.45 times (P<0.05) (Fig.2). A significant increase in IL-8 was observed after reperfusion - 7.32 times higher (P<0.05) than the values of 6-hour ischemia. The IL-10 level increased by 1.58 times (P<0.05) in relation to the initial data and did not change after reperfusion. The TF index decreased by 1.34 times (P<0.05), compared to the initial value, and by 1.93 times (P<0.05) after reperfusion.

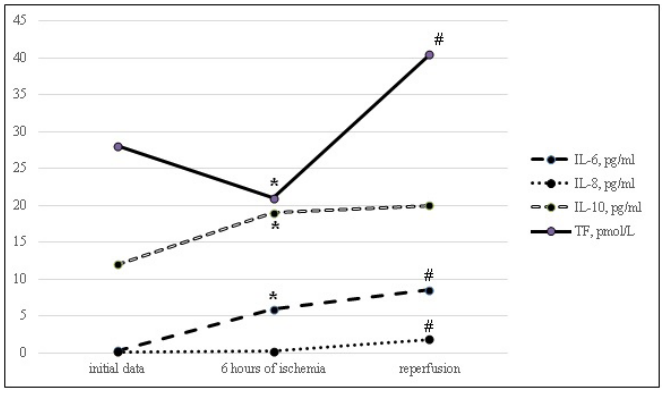

Fig. 2. *Changes in the levels of ILs and TF after 6-hour ischemia and reperfusion (* - statistically significant differences with initial data, # - statistically significant differences between 6-hour ischemia and reperfusion).*

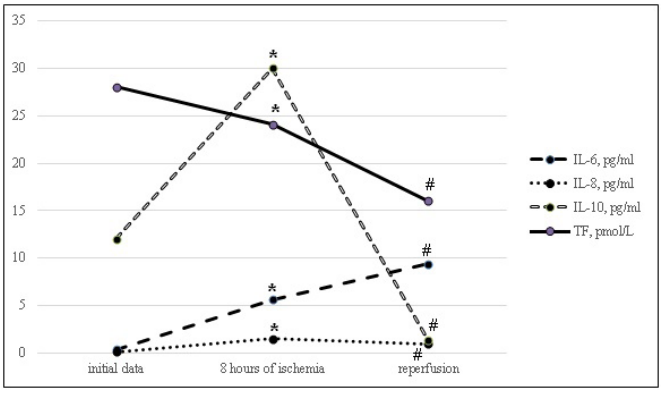

Fig. 3. *Changes in the levels of ILs and TF after 8-hour ischemia and reperfusion (* - statistically significant differences with initial data, # - statistically significant differences between 8-hour ischemia and reperfusion).*

After 8 hours of ischemia, the serum concentration of IL-6 and IL-8 increased by 16.56 times (P<0.01) and 12.33 times (P<0.05), respectively, compared to the initial data (Fig.3). At the same time, reperfusion contributed to an increase in IL-6 by 1.67 times (P<0.05) and a decrease ll in IL-8 by 1.51 times (P<0.05). The content of IL-10 increased by 2.5 times (P<0.05) from the baseline level and fell after reperfusion by 23.08 times (P<0.05) relative to the data of 8-hour ischemia. The TF index decreased by 1.16 times (P<0.05) from the initial level and by 1.5 times (P<0.05) after reperfusion.

By analogy with the processes occurring during the development of ischemia of other organs, it can be assumed that the very change in the intensity of blood flow in the ischemic tissue through the participation of mechanoreceptors can already serve as a signal to start the synthesis of proinflammatory cytokines.[5] Developing hypoxia leads to a subsequent increase in the level of oxygen radicals, osmotic disorders, and damage to cell biomembranes.[10,11-14] Thus, cytokines synthesized during the development of aseptic inflammation serve as endogenous mediators of the development of post-ischemic disorders in tissues.[15] IL-6 is synthesized by many types of cells that trigger and regulate inflammation and immune response, and has an extremely versatile biological effect.[7] The properties of IL-8 to cause cell migration and promote cell adhesion define it as an active participant in acute inflammatory response at the pathogen penetration sites.[5,6] When studying the blood plasma of rats for the IL-6 and IL-8 content, we noted their marked increase from the initial values to the eighth hour of intestinal ischemia. IL-10 is an anti-inflammatory cytokine produced by monocytes, macrophages, and activated CD4 cells. Attention is drawn to the ability of macrophages themselves to produce this cytokine, which is a strong inhibitor for them.[6] IL-10 is known to promote humoral immune response and suppress the cellular immune response, as well as being a natural inhibitor of the nuclear factor, which plays a universal role in the development of an inflammatory cascade of various etiologies.[4,5] We observed an increase in this indicator during acute mesenteric ischemia, with maximum values after 8 hours of ischemia.

TF catalyzes the conversion of the inactive plasminogen to the active plasmin and is an important component of the fibrinolysis system.[6] It is also one of the enzymes most often involved in the processes of destruction of the basement membrane, extracellular matrix, and cell invasion. The study of the TF content in the blood during the AMI simulation showed a decrease in its values during the entire period of ischemia, relative to the initial indicators, which can be explained by an increased expenditure of the factor in conditions of ischemic damage to intestinal tissues.

Next, we studied the effect of reperfusion after different periods of ischemia on the dynamics of IL-6, IL-8, IL-10, and TF indicators, depending on the time of ischemia. The recovery of mesenteric blood circulation after 3-hour ischemia, caused an increase in the levels of IL-6, IL-8, and IL-10, which indicated the predominance of anti-inflammatory cytokine potential in reperfusion at this time of ischemia. The reperfusion after 6-hour ischemia caused an increase in the levels of IL-6, IL-8, and TF. Thus,

reperfusion at this time of ischemia was characterized by a predominance of proinflammatory cytokine potential. Restoring of the mesenteric circulation after 8-hour ischemia was characterized by an increase in the IL-6 concentration and a decrease in the IL-8 concentration. It is noteworthy that the restoration of blood circulation at this time led to a sharp decrease in the IL-10 concentration, which can be explained by the severity of the pathological process. In addition, there was a decrease in the TF concentration, relative to the indicators before reperfusion, which is probably due to the expenditure of the latter in the conditions of ongoing necrotic changes in the intestine. It is also impossible to exclude the progression of mesenteric artery thrombosis, which can prevent the "leaching" of the factor into the general blood flow.

Treatment of acute mesenteric ischemiais primarily aimed at restoring blood circulation in the mesenteric bed.[16] However, although the restoration of blood flow is essential in the mitigation of ischemic damage, reperfusion can initiate a whole series of inflammatory reactions and cause a local cellular response with the inclusion of molecular mechanisms.[17] It was found that as a result of reperfusion, oxygen flow to damaged cells is restored, as a result of which the mitochondria begin to produce active oxygen metabolites, which lead to tissue damage.[18-21] Cells die not at the height of ischemia, but after complete or partial restoration of blood circulation, as a result of increased oxidative stress reactions.[22]

Thus, we found that intestinal ischemia in rats for 8 hours is accompanied by pronounced changes in the immune system and the development of a systemic inflammatory reaction syndrome, which is aggravated by reperfusion. The detected changes can serve as laboratory markers that characterize the course and stage of acute mesenteric ischemia.

Competing Interests

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

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Production, Properties and Transit of Copper-Pectic Gel Particles through an Artificial Gastroenteric Environment

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Abstract

Spherical copper-pectic gel particles (CuPGPs) were obtained from aqueous solutions of commercial apple pectin (AP) AU701 (2%, 4%) in the presence of copper ions ($CuCl_2$, 1%-10%), and their morphological (diameter) and structural-mechanical (density) characteristics were studied. It was found that with an increase in the concentration of AP from 2% to 4%, the diameter of dry gel particles at all tested concentrations of copper chloride (1-10%) increased from 0.64-0.76 mm (2% AP) to 0.87-0.94 mm (4% AP), and that the density of dry gel particles with an increase in the concentration of AP also increased from 1.27-1.48 mg/mm^3 (2% AP) to 1.42-1.55 mg/mm^3 (4% AP). The swelling and degradation of the obtained CuPGPs in an artificial gastroenteric environment was studied. It has been established that the CuPGPs based on 4% AP with 10% $CuCl_2$ have the highest degree of swelling in the acidic environment of the intestinal gastric fluid (SGF). CuPGPs, depending on the concentration of AP and copper ions, are degraded in different parts of the intestine—in simulated intestinal fluid (SIF) or simulated colonic fluid (SCF). (**International Journal of Biomedicine. 2020;10(4):421-423.**)

Key Words: apple pectin • cross-linking agents • copper ions • gel particles • gastroentestinal tract

Abbreviations

AP, apple pectin; **CuPGPs**, copper-pectic gel particles; **CaPGPs**, calcium-pectic gel particles; **GIT**, gastroentestinal tract; **SGF**, simulated gastric fluid; **SIF**, simulated intestinal fluid; **SCF**, simulated colonic fluid.

Introduction

Pectins are natural biodegradable polysaccharides that, due to their high physiological activity and ability to form gels, are widely used in the food and pharmaceutical industries.[1] The main commercial pectins with high gel-forming ability are apple and citrus pectins.[2,3] The widespread use of pectins is based on their ability to form gels. High methyl-esterified pectins (HMEPs) form gels with sugar and acid, low methyl-esterified pectins (LMEPs) quickly form gels in the presence of divalent metal ions (calcium, etc.).[4,5]

Pectin, a naturally occurring component of human food, slows down the rate of digestion, resulting in less food absorption. Due to its high water-binding capacity, pectin gives a feeling of fullness, which reduces food intake. These properties of pectin are used in the treatment of binge eating disorders.[6]

LMEPs form gels in the presence of divalent cations, with calcium ions being the most commonly used as a cross-linking agent. Modern articles and reviews have focused specifically on this type of gel, which is often referred to as "calcium gel."[3,7] Copper ions can be a promising cross-linking agent in the preparation of pectic gel particles. Copper

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is one of the most important essential trace elements that are essential for the normal life of plants and animals, including humans. It is a biogenic element, a permanent component of the human body. The main biological role of copper is that it is a component of many enzymes with redox activity.[8] Using copper ions as a cross-linking agent, the gelation of HMEPs,[9] Cu-alginate [10] has been previously studied. The study of the gelation of LMEP (AP) using copper ions as a cross-linking agent is also relevant.

The aim of our work was to obtain and study the properties of CuPGPs, and to study the swelling and degradation of CuPGPs during incubation in the artificial gastroenteric environment.

Materials and Methods

Formation of dry CuPGPs and study of morphological and structural-mechanical properties

Pectic gel particles were obtained on the basis of commercial AP AU701 (AP, Herbstreith & Fox KG, Germany) using copper ions as a cross-linking agent by the method of ionotropic gelling, which was described in earlier works.[4,5] CuPGPs were obtained at AP concentrations of 2% and 4% and $CuCl_2$ concentrations of 1%-10%. Certain weighed portions of pectins were dissolved in corresponding volumes of distilled water by slow stirring with a magnetic stirrer MM-5 (Russia) for 2-5 hours at room temperature until complete dissolution.

Gel particles of spherical shape were prepared by drop-by-drop injection of a pectin solution from a syringe through a needle with a hole diameter of 0.7 mm on the distance of 4-5 cm in the slowly stirred copper chloride solution and further stirring for 20 min at room temperature. The resulting gel particles were then washed three times in distilled water with stirring for 5 minutes and dried for 10-14 h at 37°C.

Further, the diameter, surface area, volume, and density of CuPGPs were determined using an optical microscope (Altami, Russia) with a camera and an image analysis program (ImageJ 1.46r program, National Institutes of Health, USA). For calibration, a linear scale was used; one pixel corresponded to 0.024 mm.

Study of swelling and degradation of CuPGPs in simulated gastrointestinal media

The swelling and degradation of CuPGPs were studied under conditions simulating the gastrointestinal environment: for these purposes, the simulated gastric fluid (SGF solution, pH 1.25), simulated intestinal fluid (SIF solution, pH 7.0) and simulated colonic fluid (SCF solution, pH 7.0), as described previously.[11]

To determine swelling and degradation, dry CuPGPs (1-2 mg) were placed in Petri dishes (diameter 3.5 cm) and subsequently incubated in 3 ml of SGF (2h), SIF (4h) and SCF solutions with shaking in a shaker (Titramax 1000, Heidolph, Germany) at 100 rpm and 37°C. The diameter, surface area, volume and density of 100 randomly selected gel particles were measured as described above after certain time intervals. The experiments were performed in triplicate. The degree of gel swelling (SD,%) was determined by the formula[12]: $SD = (D_1-D_0)/D_0 \times 100\%$, where D_1 – diameter of the particles (mm) after a certain incubation time in the medium, D_0 – initial diameter of the particles (mm).

The statistical analysis was performed using the statistical software BioStat (version 4.03) and Microsoft Office Excel 2007.

Results and Discussion

The gel-forming properties of pectins depend on the degree of methyl esterification of carboxyl groups of galacturonic acid residues, on the structure of pectin side chains, pectin concentration, pH, concentration of metal ions, ionic strength of solution, and temperature.[3,13] Spherical pectic gel particles are formed as a result of the gelation, in which intermolecular cross-links arise between divalent metal ions and negatively charged carboxyl groups of pectin macromolecules.[14,15]

The 2% and 4% aqueous solutions of AP in the presence of copper ions ($CuCl_2$, 1-10%) yield the spherical CuPGPs, which were subjected to determine their morphological and structural-mechanical characteristics (diameter, density).

It was found that with an increase in the concentration of AP from 2% to 4%, the diameter of dry gel particles at all tested concentrations of $CuCl_2$ increased from 0.64-0.76 mm (2% AP) to 0.87-0.94 mm (4% AP) (Table 1).

Table 1.

Diameter of dry copper-pectic gel particles (M±SD, mm)

AP concentration	$CuCl_2$ concentration					
	1%	2%	3%	4%	5%	10%
2%	0.64 ±0.03	0.65 ±0.04	0.70 ±0.04	0.72 ±0.05	0.75 ±0.03	0.76 ±0.04
4%	0.87 ±0.03	0.87 ±0.04	0.88 ±0.04	0.90 ±0.02	0.93 ±0.03	0.94 ±0.04

The density of dry gel particles with an increase in the concentration of AP at all tested concentrations of $CuCl_2$ also increased from 1.27-1.48 mg/mm^3 (2% AMP) to 1.42-1.55 mg/mm^3 (4% AP) (Table 2).

Table 2.

Density of dry copper-pectic gel particles (M±SD, mg/mm^3)

AP concentration	$CuCl_2$ concentration					
	1%	2%	3%	4%	5%	10%
2%	1.27 ±0.18	1.35 ±0.13	1.36 ±0.25	1.41 ±0.28	1.44 ±0.19	1.48 ±0.22
4%	1.42 ±0.14	1.45 ±0.12	1.48 ±0.10	1.50 ±0.10	1.52 ±0.12	1.55 ±0.18

The previously obtained dry CaPGPs based on 3% AP using calcium ions (0.34 M $CaCl_2$) as a cross-linking agent have a larger diameter of 1.21±0.05 mm and about three times

smaller density[3] – 0.51±0.05 mg/mm^3 – than CuPGPs. Seslija et al.,[9] when comparing a number of divalent cations as cross-linking agents, found that Cu^{2+} ions bind pectin most strongly and Ca^{2+} ions weakly. Probably for this reason, in our studies, copper ions formed smaller, but denser, gel particles from AP than calcium ions.

The swelling and degradation of the obtained CuPGPs in an artificial gastroenteric environment was studied. Gel particles formed from 2% AP with 1%-5% $CuCl_2$ swelled in a simulated gastric fluid SGF by 90%-150% and degraded in a simulated intestinal fluid SIF during the first 30 minutes of incubation in it. Gel particles obtained from 2% and 4% AP with 10% $CuCl_2$ swelled in SGF by 161%-173% and then, depending on the concentration of pectin, degraded in SIF or SCF (Fig.1).

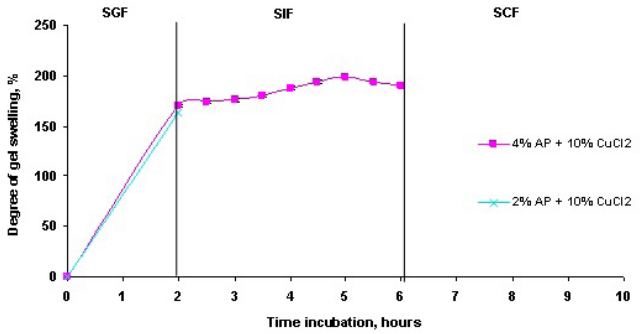

Fig.1. *Swelling and degradation of CuPGPs formed from AP (2% and 4%) and copper ions (10% $CuCl_2$) in an artificial GIT.*

Gel particles formed from 2% AP with 10% $CuCl_2$ degraded in SIF during the first 30 minutes of incubation in it. Gel particles obtained on the basis of 4% AP with 10% $CuCl_2$ are more stable and degraded in SCF in the first minutes of incubation in it.

Previously, we found that CaPGPs formed from 3% AP using calcium ions as a cross-linking agent completely degrade in a simulated fluid of the small intestine after 3h of incubation in it.[3]

Thus, the gel particles formed on the basis of 4% AP with 10% $CuCl_2$ as a cross-linking agent have the greatest degree of swelling in the acidic environment of the stomach. CuPGPs, depending on the concentration of AP and copper ions, are degraded in different parts of the intestine – in SIF or SCF. Copper ions as cross-linking agents form smaller, but denser gel particles from AP than calcium ions.

Competing Interests

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

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Gut Microbiota Shift in Obese Adolescents Born by Cesarean Section

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Abstract

Background: It is known that in the early postnatal period a variety of factors affect the gut microbiota (GM) composition, including delivery mode. The effect of delivery mode on the human GM in the late postnatal period remains unexplored. A shift of GM composition due to delivery mode may contribute to the development of obesity in adulthood.

Methods and Results: The study included six adolescents aged between 11 and 17 years treated and examined at the Clinic of the Scientific Center for Family Health and Human Reproduction (Irkutsk, Russia) in 2016. Stool samples were collected following the standard operating procedures according to the International Human Microbiome Standards. Metasequencing of V3-V4 variable regions of the 16S rRNA gene was performed by the Novogene Company (China) on the Illumina platform. Bioinformatic analysis was done by the bri-shur.com services. Sequencing reads were presented as normalized values.

In general, the GM composition of obese adolescents born by cesarean section was characterized by composition heterogeneity within the Bacteroidetes phylum and the dominance of certain phylotypes as signs of dysbiosis for each adolescent. We detected an increased abundance of phyla Bacteroides and Proteobacteria, and an absence of Tenericutes in obese adolescents born by Caesarean section. On the level of genera, the prevalence of Bacteroides and Bacteroides S24-7 phylotypes, and the absence of the RF39 phylotype, led to the GM shift associated with a cesarean section or obesity.

Conclusion: Obese adolescents born by cesarean section delivery present the shift in GM composition.(**International Journal of Biomedicine. 2020;10(4):424-429.**)

Key Words: gut microbiota • dysbiosis • amplicon metasequencing • cesarean section • vaginal birth • obesity • adolescents

Abbreviations

ALT, alanine transaminase; **BMI**, body mass index; **BW**, body weight; **FPG**, fasting plasma glucose; **GM**, gut microbiota; **HDL**, high-density lipoprotein; **LDL**, low-density lipoprotein; **SDS BMI**, standard deviation score of BMI; **TG**, triglycerides; **TC**, total cholesterol.

Introduction

The microbiota of the gastrointestinal tract constitute the complex, self-regulating microbial ecosystem that contains trillions of microorganisms. Gut bacteria play a fundamental role in human health by promoting intestinal homeostasis. It is known that a large number of factors affect the composition of a child's gut microbiota (GM), such as the mode of delivery,[1-4] the type of breastfeeding,[5,6] and antibiotic therapy.[7] During the perinatal period immediately after vaginal delivery, the infant intestine is colonized by maternal bacteria belonging to *Bacteroides*, *Bifidobacterium*, *Parabacteroides*, *Escherichia*, *Shigella,*[8] *Lactobacillus*, and *Prevotella*.[9] This colonization process is a massive influx of antigens, and it provides a physiological adaptation for infants. cesarean delivery breaks the normal colonization of the infant gut by preventing exposure to maternal microbes. The intestine of infants delivered by cesarean delivery is colonized by bacteria typical

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for the skin, such as *Staphylococcus*, *Corynebacterium*, and *Propionibacterium*,[10] and has an increase of *Clostridium difficile*. It also has a slowed growth of *Bacteroides*, *Bifidobacterium*, and *Escherichia coli*, compared to vaginally born babies.[10,11]

Multiple studies suggest that cesarean delivery has a postnatal impact on children's GM. Some of these studies show an imbalance in GM composition or dysbiosis among infants between two and six weeks of age,[1,2] whereas other data point to microbiota heterogeneity persisting up to a year,(3,4) or extending up to seven years.[12] The influence of delivery mode on the GM composition in the late postnatal period has been little studied, especially in the teenage period, as a critical period for health.

Moreover, in the last decades, GM was acknowledged as one of the key pathogenic factors affecting many components of the metabolic syndrome and obesity. Dysbiosis may contribute to a predisposition for diseases, including obesity formation.[13-15] A deep understanding of the factors provoking GM dysbiosis in lifelong health is needed.

In this study, we checked the GM composition in adolescents born by cesarean section and those born by vaginal delivery with an assessment of how that composition contributes to the development of obesity.

Materials and Methods

The study was approved by the Ethics Committee of the Scientific Center for Family Health and Human Reproduction Problems. Written informed consent was obtained from each research participant (or the participant's parent/guardian).

Exclusion criteria were intake of prebiotics or probiotics in the previous six months, antibiotic therapy in the previous three months, chronic inflammatory bowel diseases in the past, severe somatic pathologies, and intestinal infections in the last six months in anamnesis.

The study included six adolescents aged between 11 and 17 years with normal weight (SDS BMI <1.0) or obesity (SDS BMI ≥2.0)[16] treated and examined at the Clinic of the Scientific Center for Family Health and Human Reproduction (Irkutsk, Russia) in 2016. Three adolescents were born by cesarean delivery: One male adolescent was within the normal weight range (D38-m), the other two participants, male and female, had obesity (D10-m and D16-f). For comparison, three adolescents who were born vaginally were also included. Matched pairs of adolescents had the same gender, and were comparable for breastfeeding duration and age, and were born by a vaginal delivery. One male adolescent was within the normal weight range (D44-m); the other two, a male and a female, had obesity—D39-m and D12-f, respectively. The clinical characteristics of participants are presented in Table 1.

The concentration of FPG, TC, TG, HDL, LDL, and serum ALT was measured using a Mindray Automatic Biochemistry Analyzer. The reference for LDL was less than 3.36.[17] The reference for ALT was less than 25IU/l.[18] The reference of atherogenic index was less than 3.0.[19]

Stool samples were collected following the standard operating procedures according to the International Human Microbiome Standards. The quality of DNA was assessed in 1% agarose gel electrophoresis, DNA concentration was measured using the NanoDrop™ spectrophotometer (Thermo Scientific, USA). Metasequencing of V3-V4 variable regions of the 16S rRNA gene was performed by the Novogene Company (China) on the Illumina platform. Bioinformatic analysis was done by the bri-shur.com services.[20] Sequencing reads were presented as normalized values.

Table 1.
Clinical characteristics of participants

Variable	Cesarean delivery			Vaginal delivery		
	Obesity		Normal weight	Obesity		Normal weight
	D10-m	D16-f	D38-m	D39-m	D12-f	D44-m
Gender	male	female	male	male	female	male
Age, years	11	11	17	13	15	17
Breastfeeding duration >3 month	yes	yes	none	yes	yes	none
BMI, kg/m^2	30.4	33.0	21.8	29.1	37.4	22.0
SDS BMI	3.3	3.4	0.1	2.6	3.2	0.3
TC, mmol/L	4.98	4.97	4.99	4.46	4.98	3.00
TAG, mmol/L	0.98	0.89	1.14	1.24	0.85	0.45
HDL, mmol/L	1.16	1.52	1.50	1.00	1.72	NA
LDL,mmol/L	3.37*	3.04	2.97	2.90	2.87	NA
Atherogenic index	3.3*	2.3	2.3	3.5*	1.9	NA
FPG, mmol/L	4.8	4.0	4.1	5.0	5.5	3.9
ALT, IU/L	26.0*	24.8	13.8	23.4	12.6	27.4

*Note: * - above the reference, NA – not available data*

Results and Discussion

GM composition of adolescents at the phylum and class level

We found changes in GM composition for adolescents, depending on BW or delivery mode. We have seen the shift of GM composition in obese adolescents born by cesarean section already at the high taxonomic level. Compared with obese adolescents who were delivered vaginally, those born by cesarean delivery had bacterial communities with a higher abundance of the phyla Bacteroidetes and Proteobacteria, and a lower abundance of Firmicutes and absence of Tenericutes (Table 2). Other phyla, such as Actinobacteria and Verrucomicrobia, were represented in a similar abundance for adolescents with different BW who had been delivered via a different mode.

At the level of classes and genera, the shift in GM composition was found to be more prominent. Obese adolescents born by cesarean section had an increased abundance of the *Bacteroidia* class (the Bacteroidetes phylum) and a decrease of *Clostridia* (the Firmicutes phylum). Figure 1 shows the top 25 most abundant genera of the individual gut microbiomes of adolescents born by vaginal (A) and cesarean (B) delivery. All three vaginally born adolescents, regardless of BW, shared bacterial genera belonging to *Clostridia*, *Bacteroidia*, *Gammaproteobacteria*, *Betaproteobacteria*, and *Deltaproteobacteria* classes. They

formed the common bacterial core. The GM composition of adolescents born by cesarean section was different for each of them without the bacterial core. This fact illustrates how the mode of delivery is essential for GM formation.

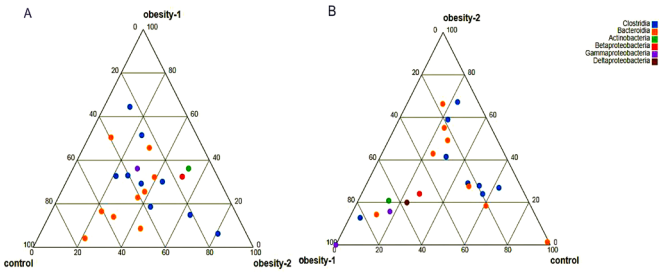

Fig. 1. *The top 25 most abundant genera of the individual gut microbiomes of adolescents born by vaginal (A) and cesarean (B) delivery.*

Note: obesity-1 and obesity-2 – obese adolescents, control – normal-weight adolescents

Bacteroidetes, Firmicutes, and Proteobacteria are the three most abundant bacterial phyla of adult GM. Their ratio is a very important sign of healthy GM composition. This finding does not concur with previous metagenomic studies that demonstrated a lower amount of Bacteroidetes and a higher amount of Firmicutes for obese people than for lean controls.[21] Such contradictions can be explained by including in our study the delivery mode as the factor influencing GM composition. Regarding Proteobacteria, especially the *Gammaproteobacteria* class, on the one hand, its members such as enterococci and *E. coli* are part of the healthy GM composition;[22] on the other hand, many opportunistic microorganisms belong to this class.[23] Therefore, very often, an increased abundance of *Gammaproteobacteria* was found to be associated with gut dysbiosis.

Other phyla are presented in GM composition in a considerably smaller amount, but nonetheless play an important role in the GM metabolism. Actinobacteria and Verrucomicrobia belong to the healthy GM composition, and they have

Table 2.

Abundance of dominant phyla, classes and their ratios depending on delivery mode and body weight

Taxonomic level	Cesarean delivery			Vaginal delivery		
	Obesity		Normal weight	Obesity		Normal weight
	D10-m	D16-f	D38-m	D39-m	D12-f	D44-m
Phylum						
Bacteroidetes	63.4	61.3	49.9	62.8	53.7	53.1
Firmicutes	19.7	35.2	45.6	33.0	40.6	41.5
Proteobacteria	13.9	2.5	2.8	3.3	4.2	2.3
Actinobacteria	1.5	0.7	0.7	0.4	1.4	1.1
Verrucomicrobia	0.3	0.3	0.2	0.4	0.1	0.2
Tenericutes	0	0	0.8	0.1	0	1.7
Ratios for phyla						
Bacteroidetes/Firmicutes	3.2	1.7	1.1	1.9	1.3	1.3
Proteobacteria/Actinobacteria	9.3	3.6	4.0	8.3	3.0	2.1
Class (phylum)						
Bacteroidia (Bacteroidetes)	63.4	61.3	49.9	62.8	53.7	53.1
Clostridia (Firmicutes)	18.6	34.6	45.0	32.8	40.0	40.4
Bacilli (Firmicutes)	1.1	0.1	0.1	0.1	0.3	0.4
Erysipelotrichi (Firmicutes)	0.1	0.4	0.4	0.2	0.3	0.8
Gammaproteobacteria (Proteobacteria)	11.1	1.2	1.4	1.4	2.3	1.0
Betaproteobacteria (Proteobacteria)	1.6	0.8	1.0	0.9	1.2	0.3
Deltaproteobacteria (Proteobacteria)	1.0	0.5	0.5	1.0	0.7	0.9
Actinobacteria (Actinobacteria)	1.1	0.4	0.3	0.2	1.0	0.4
Coriobacteriia (Actinobacteria)	0.4	0.4	0.4	0.2	0.4	0.7
Ratios for classes						
Bacteroidia/Clostridia	3.4	1.8	1.1	1.9	1.3	1.3
Gammaproteobacteria/ Actinobacteria	10.1	3.0	4.7	7.0	2.3	2.5
Clostridia/Bacilli	16.9	346.0	450.0	328.0	133.3	101.0
Clostridia/Erysipelotrichi	186.0	86.5	112.5	164.0	133.3	50.5
Bacteroidia/Bacilli	57.6	613.0	499.0	628.0	179.0	132.8
Bacteroidia/Erysipelotrichi	634.0	153.3	124.8	314.0	179.0	66.4
Betaproteobacteria/ Actinobacteria	1.5	2.0	3.3	4.5	1.2	0.8
Deltaproteobacteria/ Actinobacteria	0.9	1.3	1.7	5.0	0.7	2.3

Note: m – male, f – female

Bifidobacterium and *Akkermansia* as their members.[24,25] We did not observe differences for these phyla between adolescents born vaginally and those born by cesarean section, which probably can be explained by the fact that these phyla are balanced by others. However, our study detected no bacteria belonging to the Tenericutes phylum for obese adolescents born by cesarean section. Tenericutes play an essential role in the host metabolism as intracellular microorganisms and may change intracellular metabolism.[26] Contradictions between the results of current findings and those of previous studies can be explained by including the delivery mode as a factor influencing GM composition.

GM composition of adolescents at the phylotype level

Taxonomic-level differences in GM composition were found for several phylotypes. Table 3 shows the most abundant 20 bacterial phylotypes in the gut microbiome of adolescents with different delivery mode and BW. In general, the GM composition of obese adolescents born by cesarean section was characterized by composition heterogeneity within the Bacteroidetes phylum and the dominance of certain phylotypes as signs of dysbiosis for each adolescent.

Table 3.

The top 20 most abundant bacterial phylotypes of adolescents with different delivery mode and body weight

Phylotype	Cesarean delivery			Vaginal delivery		
	Obesity		NW	Obesity		NW
	D10-m	D16-f	D38-m	D39-m	D12-f	D44-m
Bacteroidetes						
Prevotella	47.4	9.2	7.6	40.1	26.3	21.4
Bacteroides	11.5	44.8	11.3	14.3	17.3	12.8
PL_6_Bacteroides S24-7*	0.2	0.3	23.7	1.7	2.4	6.4
PL_5_Rikenellaceae	1.3	1.4	2.5	0.9	1.8	3.5
PL_4_Bacteroidales	0.1	0.1	0.1	0.1	0.6	2.2
PL_6_[Prevotella]	0.5	0.7	0.4	2.3	0.5	1.8
Parabacteroides	1.1	2.3	1.3	1.2	1.8	1.8
PL_5_[Barnesiellaceae]	0.6	1.6	0.7	0.8	1.3	1.5
Firmicutes						
PL_5_Ruminococcaceae	5.3	6.5	15.3	11	8.9	13.6
PL_5_Lachnospiraceae	1.4	9.6	3.3	3.3	7.8	6.6
PL_4_Clostridiales	2.1	5.4	12.5	4.6	5.5	5.8
Dialister	1.1	3.5	1.4	2.9	1.9	4.1
Faecalibacterium	1.2	1.9	1.4	1.4	6	2
Ruminococcus	1.6	2.3	4.4	2.1	3.1	1.9
Oscillospira	1.2	1.5	2.3	2.2	1	1.1
Megasphaera	2.3	0.4	0.1	2.2	0.4	0.8
Proteobacteria						
PL_5_Enterobacteriaceae	4.8	11	1.3	1.3	2.1	0.7
Sutterella	1.5	0.8	0.8	0.8	1.2	0.2
PL_5_Xanthomonadaceae	5.6	0	0	0	0	0
Tenericutes						
PL_6_RF39	0	0	0.7	0.1	0	1.6

*Note: NW -Normal weight; m – male, f – female, * PL – phylotype level – the level of identification, 4 – the order, 5 – the family, 6 – the genus*

As others have reported, we observed that vaginally born adolescents had a slightly higher representation of the *Bacteroides* genus than the cesarean born. Thus, Madan et al. showed vaginal delivery is associated with an increased number of *Bacteroides* (P=0.0007, q=0.02) for infants.[2] In addition, the obese female adolescent born by cesarean section has twice the increase of *Bacteroides* as adolescents born by vaginal delivery; this is in contradiction to some research reports showing an absence of *Bacteroides* for infants born by cesarean delivery.[3] Present controversies may be explained by a difference in age among participants. GM of adolescents must be more stable and adult-like than it is during the early childhood period. However, according to our results those who were born by cesarean delivery still present the shift in bacterial composition.

Another huge inequality was found for the phylotype Bacteroides S24-7, which has structural similarities to *Bacteroides* genus. This phylotype was considerably more represented in GM of normal weight adolescents than *Bacteroides* itself. In contrast, the obese adolescent compensates for the decrease in Bacteroides S24-7 by an increase in Lachnospiraceae phylotype (the Firmicutes phylum). This evidence is in agreement with the data suggesting the participation of Firmicutes in the deposition of fats.

The GM heterogeneity is characterized by an appearance of new members of a bacterial community. Against this background some community members may get lost. For example, GM of obese adolescents born by cesarean section was characterized by an absence of the RF39 phylotype (the Tenericutes phylum).

In contrast, samples of vaginally born adolescents were homogeneous in the Bacteroidetes phylum, which indicates a stable healthy GM state. Therefore, the normal weight adolescents born by vaginal delivery showed increased values for all the most abundant phylotypes of the Bacteroidetes phylum equally (Table 3), which confirms the literature data that shows an increased abundance of the members of phylum Bacteroidetes in normal weight children.[21]

Therefore, the increased abundance of separate phylotypes among the top 20 most abundant bacterial phylotypes may indicate that GM participates in the formation of obesity among adolescents born by cesarean section.

Features of the metabolic pathways in GM adolescents

The association of microbial and host metabolism is well known;[27] thus, an imbalance in the gut microbiome may contribute to metabolic changes in the host. We made a model of the metabolic pathways of adolescent gut microbiomes based on a dataset of paired interactions (according to the present/absent principle) among the 25 most represented phylotypes (Figure 2). The metabolic pathways of gut microbiomes cross each other for all three adolescents born vaginally. However, the metabolic pathways of gut bacteria of three adolescents born by cesarean section are completely autonomous, unique,

and do not have common contact points. Later we analyzed biochemical parameters of obese adolescents born by cesarean section and found some values above the reference numbers (Table 1). LDL of the male adolescent with obesity was 3.37 mmol/L, and ALT was 260 IU/L. In addition, the atherogenic index of male adolescents with obesity was increased regardless of delivery mode. It is likely that the metabolism of adolescents born by cesarean section can be obesogenic due to altered GM composition, but the concrete metabolic pathways underlying these observations remain unknown.

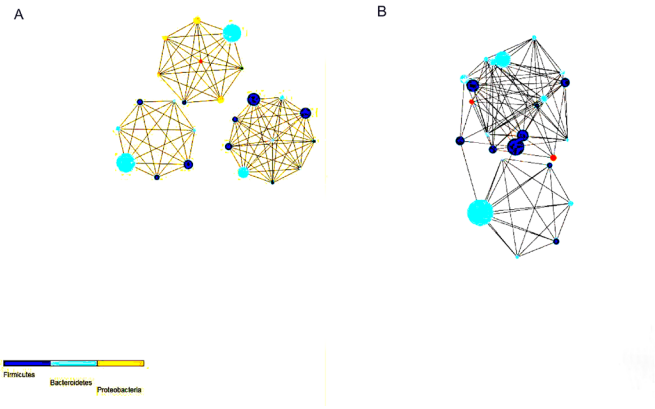

Fig. 2. *Visualization of the possible metabolic pathways of the 25 most represented phylotypes of gut microbiomes of adolescents born by cesarean (A) and vaginal (B) delivery.*
Note: two cases with obesity stick together, the third one with normal weight lies apart from them

In conclusion, understanding the factors provoking GM dysbiosis in adolescents is critical for determining at an early stage of life any predisposition to certain illnesses, namely obesity. To this end, using the metagenome analysis, we showed a shift in the GM composition of obese adolescents born by cesarean section both at the level of large taxa and at the level of lower taxonomic units. We described the shift for adolescents, as opposed to numerous studies showing changes in the microbiome of young children. This fact makes our study important and helpful for understanding the mechanisms of keeping GM dysbiosis at later stage of life.

Competing Interests

The authors declare that they have no competing interests.

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CASE REPORT

Imaging Features of Cervical Spinal Motion Segment Lesions before and after Surgery: A Case Report

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Abstract

Spinal injuries remain a serious modern health problem that leads to a high level of disability and prehospital mortality. This clinical case of cervical fracture-dislocation demonstrates the diagnostic signs and the surgical approach. (**International Journal of Biomedicine. 2020;10(4):430-432.**)

Key Words: spinal injury • spinal cord injury • radiology • surgical treatment

Introduction

According to a number of authors, spinal injuries amount to 1.0%-8.0% of closed trauma and 5.0%-20.0% of total musculoskeletal lesions. Spinal and spinal cord injuries demonstrate an extremely increasing trend in frequency over the past 100 years. In Russia, 10,000 cases are reported annually.[1-3] Spinal and spinal cord injuries are the third leading trauma localization despite all the preventative measures (safe working conditions, vehicle security, etc.). Cervical spine involvement was found in 12.0% of spinal injuries. When the spinal cord is affected, fatality rates in cervical injuries range from 75.0% to 80.0%.[4-6]

Acute spinal and spinal cord trauma range from 2.0% to 3.0% among all neurosurgical patients; frequently (40%-60%) they occur in combination with other bodily damage.[1,4] Concomitant spinal and spinal cord injuries constituted nearly 75.0% of these patients; in these cases, mortality is 4 times higher than in patients with an isolated injury. People suffering these injuries are predominantly males (the sex ratio is 3 males to 1 female) of working age (80%). The most common cause of spinal and spinal cord injuries is car accidents (27%-43%).[6,7]

The mortality generally depends on the severity of the spinal cord injury: 37.0% of injured persons die at the prehospital stage. The disability ranges from 50.0% to 95.0%, sometimes amounting to 100%.[3,8]

Early full detection of the pathomorphological changes in spinal lesions predetermines an effective treatment based on the pathogenetic approach. Developments in radiology and new surgical techniques point to the need for deep knowledge of spinal anatomy and biomechanics. An adequate interpretation of radiological spinal anatomy, both normal and in cases of lesions, is a topical medical issue.[9]

From the point of view of anatomy and biomechanics, facet joints and intervertebral disks are part of an entity called the spinal motion segment, the three-joint complex (the functional spinal unit (FSU)). Every vertebra belongs to 2 spinal motion segments as an upper part for the lower segment and a lower part for the upper one. Every underlying vertebra is a «transmission» for the overlying one. Nucleo-articular axes allow vertebrae to perform difficult movement as a result of the addition of simple movements around 3 mutually perpendicular axes (vertical, sagittal, and frontal).[2,10]

Each motion segment has an instantaneous axis of rotation (IAR), which is a dynamic point about which the FSU rotates and is dependent upon spinal alignment and forces acting on the spine. There are 12 potential movements about the IAR due to rotation around the three axes (x, y, and z) that pass through the center of rotation.[11]

The treatment of spinal and spinal cord lesions is a severe problem of modern neurosurgery. In most developing countries, there is no contemporary system of urgent, highly specialized medical assistance for these patients.[4] First, an orthopedic

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aspect (a restoration of bearing and protective functions of the spine) should be determined; second, a neurosurgical aspect (a spinal cord restoration). Full-fledged equipment of hospitals with diagnostic machines and with modern implantable metal constructions and adequate funding is essential.

Case Presentation

A 34-year-old white man presented to the neurosurgery department of Irkutsk Scientific Center of Surgery and Traumatology with a complaint of neck pain and reduced cervical range of motion, left hand pain (the lateral surface of the shoulder, elbow joint, and forearm), and numbness in 4-5 fingers.

Anamnesis: a victim of a car accident, this patient felt acute neck pain, reduced *cervical range of motion*, and pain and weakness in the left hand. He was taken to the fracture clinic where he was diagnosed with a closed cervical injury, C5 fracture-dislocation, and left side radiculoneuritis in C7. The patient was transferred to the neurosurgical department of Irkutsk Scientific Center of Surgery and Traumatology for special treatment.

The patient was fully conscious and hemodynamically stable. Neurological status: the pupils are equal in size, the reaction to light is brisk, and the amount of eye movement is full. The nasolabial folds are equal, the tongue is at the middle line, the pharyngeal reflex is present, and phonation is good. The tendon reflexes on the arms are brisk, ulnar and radiocarpal reflexes are weak, moderate hypoesthesia in the left lateral forearm surface and in 4-5 fingers. The strength of the left wrist is reduced to 2 points. *Status localis*: painful palpation in *processus spinosus* of C5-C7, a muscular tonic syndrome.

The cervical spine X-ray showed (Fig.1) a loss of normal lordosis with a kyphosis of the lower cervical spine; sloped anterior superior part of C5 vertebral body, which is displaced anteriorly for 0.3 cm. There was an oblique radiolucent line in the right arcus of C5 without fragment dislocation. These radiological signs were visualized: subchondral endplates osteosclerosis, formation of osteophytes, a decrease in the height of the intervertebral discs in a caudal direction, especially in C5-C7; elongation and sharpening of *processus uncinatus*. The axis is inclined to the right side without vertebral body rotation with a turn of the head.

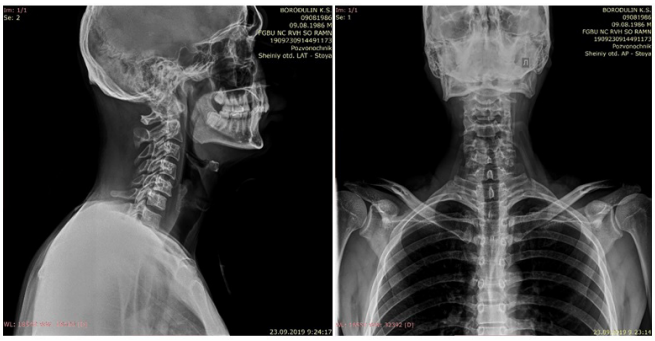

Fig. 1. *Cervical spine X-ray. Anterior C5 subluxation. C5 compression fracture (A1). Fracture of the right C5 arcus. Cervical osteochondrosis, stage III. Uncovertebral arthrosis. Spondylosis, grade II.*

A clinical diagnosis was determined: Closed spinal and spinal cord injury. C5 fracture-dislocation, interlocking dislocation of the left articular process in C5-C6, left side radiculoneuritis in C7. Pain and muscular tonic syndrome.

A surgical procedure was performed under endotracheal anesthesia, with the patient lying on his back and with a small head rotation to the right. After disinfection of the surgical field, the surgeons performed a skin incision and used the layer left parapharyngeal access to the anterior surface of the C5-C7 spinal motion segment. The rupture of the *annulus fibrosus* and pathological anterior dislocation of the C5 body (left side) were observed. There was pathological mobility during palpation of this segment. The surgeons dissected the annulus fibrous and removed the matter of the disc up to the posterior part of the *annulus fibrosus* with its tear and herniation in the vertebral canal. The C5 dislocation was reduced with a retractor, a ceramic cage-implant was fixed in moderate axis traction of cervical vertebrae. Thus, the spinal motion segment was stable. Intraoperative X-ray, hemostasis, and wound closure layer-by-layer were performed. After surgery outcomes are presented in X-ray and MSCT (Fig.2 and 3).

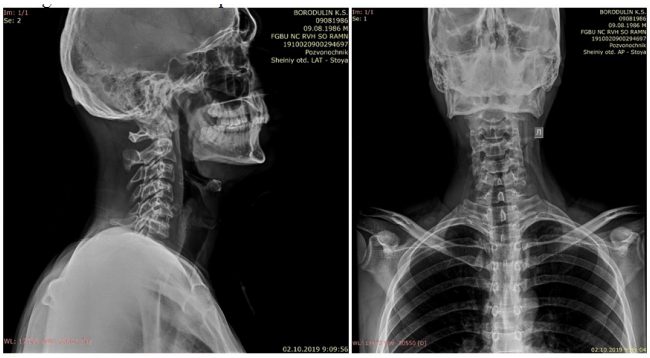

Fig. 2. *Cervical spine X-ray. After surgery. Cage-implant at the C5-C7 level. The C5 body is wedge-shape, 1.3×1.6 cm. Anterior superior parts of the C5 body are sloped; there are the osteophytes. The subluxation has been reduced. The loss of normal lordosis. The decrease of the intervertebral discs' height in a caudal direction. The axis is satisfactory.*

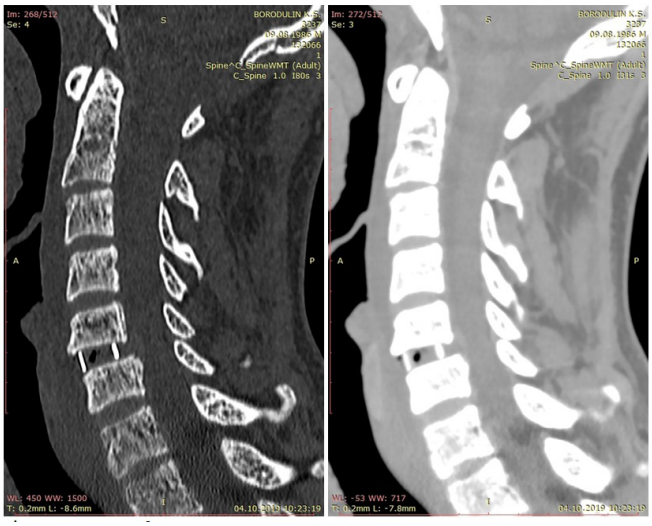

Fig. 3. *MSCT. After surgery.*

During the postoperative period, the patient was in a satisfactory state; he was active with cervical orthosis the following day. Neurological status: the pain syndrome regressed, the sensitivity in the left forearm and wrist appeared. The strength of the left wrist was 4 points. The patient was discharged from the hospital in a satisfactory state on the tenth postoperative day. The wound healing was primary.

Control check-up in 6 months (Fig.4): There were no complaints. The patient got down to work in 2 months after he was discharged from the hospital. Neurological status: there were no focal symptoms. Cervical motion to the left was slightly restricted. Palpation of *processes spinosus* was painless. The tendon reflexes on the arms were brisk, equal. The patient has been managed in accordance with clinical recommendations and protocols at the place of residence.

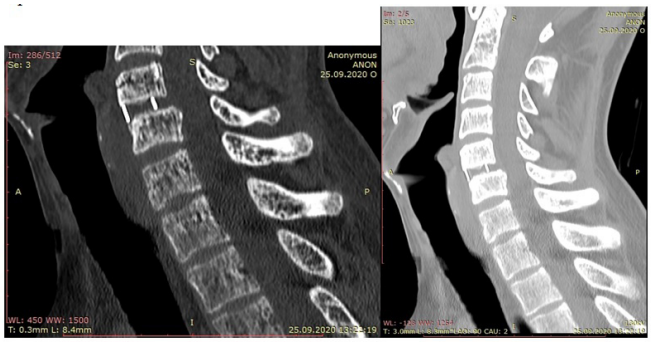

Fig. 4. *Control MSCT in 6 months after surgery. The cage-implant is present at the C5-C6 level. Consolidated fracture of left upper articular process of C6. Ante-spondylolisthesis C5, grade 1. Retro-spondylolisthesis C6, grade 1. Spondylosis at the C6 level with local stenosis of vertebral canal. Cervical osteochondrosis, stage II.*

In conclusion, the patients with cervical fracture-dislocation mostly need a surgical correction. The aims are restoration of the anatomical integrity of the spinal motion segment and resumption of the intact spinal cord. The cage-implant provides anatomical spinal integrity, vertebromedullary conflict prevention, and the reliable stabilization of the injured spine.

Competing Interests

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Population Genetics

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The structure of Haplotypes and Diplotypes in the *PNPLA3* gene in the Yakut Population

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Abstract

Background: The first GWAS searching for such genetic factors identified the *PNPLA3* gene as a major genetic determinant for the predisposition to nonalcoholic fatty liver disease in Hispanic, African American, and European American populations, according to liver fat contents, a finding that was subsequently confirmed by liver biopsy in Europeans and Asians. The aim of our research was to study the distribution of alleles, genotypes, haplotypes and diplotypes of polymorphic variants of the *PNPLA3* gene (rs2294918 and rs738409) in Yakuts.

Methods and Results: The *PNPLA3* SNPs (rs2294918 and rs738409) were analyzed by PCR-RFLP reaction. The *PNPLA3* rs738409 SNP in the Yakut population is characterized by a high frequency of the risk G allele (72%). According to the *PNPLA3* rs2294918 SNP, which suppresses the negative effect of rs738409, the protective *PNPLA3* (rs2294918) A allele was found only in 10.7% of study subjects.

Analysis of the distribution of the frequency of genotypes in the studied sample of Yakuts showed the predominance of the carriage of the *PNPLA3* rs738409 GG genotype (57.3%) and the *PNPLA3* rs2294918 GG genotype (80.7%). The frequency of the *PNPLA3* (rs2294918) AA and AG genotypes was 2.0% and 17.3%, respectively. The Yakuts often have two diplotypes [GG]-[GG] and [CG]-[GG]. Both diplotypes carry the *PNPLA3* rs738409 G allele (45.3% and 28%) and do not carry the *PNPLA3* rs2294918 A allele. The high frequency of the [GG]-[GG] and [CG]-[GG] diplotypes in Yakuts (45.3% and 25%, respectively), carrying mutant alleles G (rs738409) and not carrying the A allele (rs2294918), indicates that these diplotypes were probably adaptively favorable to the Yakuts.

Conclusion: The analysis of haplotypes and diplotypes based on the markers rs738409 and rs2294918 of the *PNPLA3* gene may contribute to future new biomarkers for the diagnosis of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis, as well as provide fundamental knowledge on human adaptation to cold. (**International Journal of Biomedicine. 2020;10(4):433-437.**)

Key Words: haplotype • diplotype • Yakut • *PNPLA3* • rs738409 • rs2294918

Abbreviations

NAFLD, nonalcoholic fatty liver disease; **PNPLA3**, patatin-like phospholipase domain-containing protein 3; **GWAS**, genome-wide association study; **SNP**, single nucleotide polymorphism; **HWE**, Hardy–Weinberg Equilibrium; **NASH**, nonalcoholic steatohepatitis.

Introduction

Maintaining body temperature has been relevant for almost the entire history of mankind, especially in the northern regions of the planet. But in the modern world, even in the north, people live all year round in warm houses, dress in warm clothes, and are exposed to the minimum effect of cold on the body. The hottest organ in our body is the liver. It heats up from 37.8°C to 38.5°C. This difference is due to the tasks that it performs. The liver is the central chemical laboratory in the

body, responsible for the production of digestive bile, filtration of the blood, and the processing of raw materials supplied with food into the necessary chemical elements for the work of other organs. However, in addition to the liver, there are two other most intense heat sources: muscles and brown adipose tissue. Brown fat is a major site of immobile thermogenesis, but other tissues, especially muscle and liver, can help adapt to the cold. Simcox et al.[1] demonstrated in mice exposed to cold that the acylcarnitines produced by the liver are required to maintain thermogenesis. Maintaining body weight requires balancing energy from food intake with energy expenditure.

In vitro studies have shown that PNPLA3 acts as an acyltransferase, which catalyzes the conversion of lysophosphatidic acid to phosphatidic acid, while the p.I148M variant reduces the enzyme activity.[2,3] At the same time, the physiological function of PNPLA3 is not fully understood. It remains unclear, for example, what is the role of changes in enzyme activity in the mechanism that forms hepatic steatosis.[4,5]

The first GWAS searching for such genetic factors identified the *PNPLA3* gene as a major genetic determinant for the predisposition to NAFLD in Hispanic, African American, and European American populations, according to liver fat contents,[6] a finding that was subsequently confirmed by liver biopsy in Europeans and Asians. The most prominent variant is the *PNPLA3* rs738409 [G], which is a nonsynonymous substitution of cytosine for guanine (C>G) that changes codon 148 from encoding isoleucine (I) to methionine (M) (I>M, I148M).[6,7]

Recently completed studies have shown that PNPLA3 can participate in the remodeling of lipid droplets in hepatocytes, leading to the accumulation of p.I148M variant protein on the surface of these droplets by disrupting ubiquitination (a decrease in the amount of ubiquitinated PNPLA3 against the background of inhibition of the bortezomib proteosome makes it difficult to release triglyceride triglycerides) and reducing the hydrolysis of triglycerides by lipases.[8,9] This hypothesis supports the conclusion that the mutant rs2294918 polymorphism of the *PNPLA3* gene decreases the expression of PNPLA3 and may weaken the effect of p.I148M, thereby reducing the risk of developing steatosis and steatohepatitis. At the same time, the *PNPLA3* E434K (the rs2294918 G>A polymorphism) variant does not affect the functional ability of PNPLA3 to reduce the content of intracellular fat in hepatocytes.[10]

It is also interesting that the *PNPLA3* p.I148M variant is present in stellate cells, and the mutant allele activates stellate cells regardless of its role in hepatocytes.[11] In addition to inducing an accumulation of triglycerides in hepatocytes, the *PNPLA3* p.I148M variant increases the risk of progression of fatty liver disease.[12] It was also shown that the *PNPLA3* p.I148M variant was associated with a higher level of alanine aminotransferase.[6] The combination of the *PNPLA3* p.I148M variant, an increased level of aspartate aminotransferase, and a high level of fasting insulin became an accurate predictor of histologically confirmed, nonalcoholic steatohepatitis in patients with NAFLD.[13] Later, it was found that the *PNPLA3* p.I148M variant is associated with the development of nonalcoholic steatohepatitis, liver fibrosis, and hepatocellular carcinoma.[14]

We previously found a high frequency of the *PNPLA3* (rs738409) [G] allele in the Yakut population (73%).[15] The accumulation of triglycerides in hepatocytes, associated with the *PNPLA3* p.I148M variant, was probably an adaptation to a cold climate; this accumulation is not needed in the modern world, but leads to NAFLD among the Yakut population.

The aim of our research was to study the distribution of alleles, genotypes, haplotypes and diplotypes of polymorphic variants of the *PNPLA3* gene (rs2294918 and rs738409) in Yakuts.

Materials and Methods

The study of the *PNPLA3* SNPs (rs2294918 and rs738409) was carried out in the Department of Molecular Genetics at YSC CMP. For the study, we used DNA samples from the collection of biomaterials of the YSC CMP (Project "The Genome of Yakutia"; No. USE_507512). A total of 150 DNA samples from healthy volunteers were examined. The inclusion criteria for the study were Yakuts by ethnicity, living in Yakutia, without liver damage by chronic viral hepatitis. Exclusion criteria: autoimmune hepatitis, primary biliary cholangitis, primary sclerosing cholangitis, hereditary hemochromatosis, Wilson-Konovalov disease, and alcohol abuse (>30g/l). For comparison, data from the 1000 Genomes project were used for the populations of Peruvians (n=85), Mexicans (n=64), Europeans from Utah in the United States (n=99), Japanese (n=104) and Yoruba tribes (n=108).[16]

Genomic DNA samples were isolated from peripheral blood lymphocytes using a commercial DNA kit, Excel Biotech (Yakutsk). The *PNPLA3* SNPs (rs2294918 and rs738409) were analyzed by PCR-RFLP reaction. The gene region containing the polymorphic variant was amplified with standard pairs of primers produced by Biotech-Industry LLC (Moscow, Russia). The reaction mixture (25 μl) for PCR contained of forward and reverse primer (Moscow, Russia), Dream Taq PCR master mix (12.5 μl), deionized water (9.5 μl), and DNA in the amount of 100 μg/ml (1 μl). A mixture for RFLP (20 μL) consisted of amplificate (7 μL), deionized water (10.9 μL), restriction buffer (2 μL), and the corresponding restriction endonucleases (2 e.a.).

PCR products were detected by horizontal electrophoresis in a 2% agarose gel plate with the addition of ethidium bromide, a specific intercalating fluorescent DNA (RNA) dye, using a standard Tris-acetate buffer at a field voltage of ~ 20V/cm for 30 minutes. RFLP products were detected by horizontal electrophoresis in 4% agarose gel stained with ethidium bromide using a standard Tris-acetate buffer at 120V for 1 hour. [15]

The study was approved by the Ethics Committee of the Yakut Science Center of Complex Medical Problems (YSC CMP). Written informed consent was obtained from each research participant (or the participant's parent/guardian).

Statistical analysis was performed using Microsoft Excel 2010. The correspondence of the distributions of genotypes to the expected values at HWE and comparison of the frequencies of allelic variants/genotypes were performed using the chi-square test. Possible haplotype variants were calculated using the Punnett square (Table 1). The haplotype frequency was determined using the EM algorithm. Linkage

disequilibrium (LD) between SNP pairs was calculated using Lewontin's coefficient D´ and Pearson's r^2 coefficient. Using the solid spine LD algorithm, a block structure was built (D´>0.75). Haploview 4.2 software was used to construct the *PNPLA3* haplotypes and frequencies based on genotyping data and to perform association tests between alleles and haplotypes of the *PNPLA3* gene.[17]

Table 1.

Possible configurations (haplotypes) at two loci of the PNPLA3 gene (shown in the Punnett square)

PNPLA3	Locus (SNP)	rs738409		
Locus (SNP)	Genotype	CC	CG	GG
rs2294918	AA	CA	CA, GA	GA
	AG	CA, CG	GA, CA, CG, GG	GG, GA
	GG	CG	GG, CG	GG

Results and Discussion

The *PNPLA3* rs738409 SNP in the Yakut population is characterized by a high frequency of the risk G allele (72%) (Table 2). The data from open sources of the 1000 Genomes project[10] reveal that the *PNPLA3* (rs738409) [G] allele is also found with a high frequency in populations of Central and South America (Peruvians - 71.8%, Mexicans - 55.5%, Colombians - 41%). Attention is drawn to the fact that the higher the G allele frequency, the higher the percentage of indigenous people in the population. For example, in the Puerto Rican population, where the indigenous Indian population is 11%, the frequency of the G allele is 31.7%. In Europeans, the frequency of the G allele is on average 22.6%. Among Asians, the Japanese have a high frequency of the G allele (42.3%). The owners of the lowest frequency of the G allele are Africans (8.6%-17.2%)

Analysis of the distribution of the frequency of genotypes of the *PNPLA3* rs738409 SNP, according to the open database of the 1000 Genomes project,[10] showed that the G allele is found with a high frequency in all the world populations. For example, the average frequency of the G allele in African populations is 89.6%, in the populations of Central and South America - 78.8%, in the populations of East Asians (Chinese, Japanese, and Vietnamese) - 81.8%, among Europeans - 62.9%, and in South Asians (Indians and Pakistanis) - 77.2%.

According to the *PNPLA3* rs2294918 SNP, which suppresses the negative effect of rs738409, the protective the *PNPLA3* (rs2294918) A allele was found only in 10.7% of study subjects. According to the 1000 Genomes project,[10] the *PNPLA3* (rs2294918) A allele is more common in Europeans than in other populations (32.3%).

Table 2.

Frequency of variants and distribution of the missense mutations in the PNPLA3 gene in the Yakut population and in the populations from the "1000 Genomes" project

Population	SNP	NC	MAF	Protein Change	Risk Allele	ObsH	PredH	HWE *P*-value
YKT (n=150)	rs738409	G:C	0.720 (G)	I148M	G (148M)	0.293	0.403	0.0019
	rs2294918	G:A	0.107 (A)	E434K	G (434E)	0.173	0.191	0.4386
PEL (n=85)	rs738409	G:C	0.718 (G)	I148M	G (148M)	0.376	0.405	0.6473
	rs2294918	G:A	0.100 (A)	E434K	G (434E)	0.153	0.180	0.3655
MXL (n=64)	rs738409	G:C	0.555 (G)	I148M	G (148M)	0.422	0.494	0.3260
	rs2294918	G:A	0.172 (A)	E434K	G (434E)	0.312	0.285	0.8301
JPT (n=104)	rs738409	G:C	0.418 (G)	I148M	G (148M)	0.394	0.487	0.0736
	rs2294918	G:A	0.087 (A)	E434K	G (434E)	0.135	0.158	0.3183
CEU (n=99)	rs738409	G:C	0.217 (G)	I148M	G (148M)	0.354	0.340	0.9869
	rs2294918	G:A	0.323 (A)	E434K	G (434E)	0.438	0.438	0.8946
YRI (n=108)	rs738409	G:C	0.116 (G)	I148M	G (148M)	0.213	0.205	1.0
	rs2294918	G:A	0.097 (A)	E434K	G (434E)	0.157	0.176	0.5046

Abbreviations: NC, nucleotide substitution; MAF, minor allele frequency; ObsH, observed heterozygosity; PredH, predicted heterozygosity; YKT, Yakuts from Yakutia, Russia; PEL, Peruvians from Lima, Peru; MXL, Mexican Ancestry from Los Angeles, USA; JPT, Japanese in Tokyo, Japan; CEU, Utah Residents (CEPH) with Northern and Western European Ancestry; YRI, Yoruba in Ibadan, Nigeria.

A weak LD was observed between the two SNPs (D´=0.096, r^2=0.003 in Yakuts; D´=0.48, r^2=0.003 in Yoruba) (Fig. 1). In other samples, strong linkage was observed (D´=1, r^2=0.282 in Peruvians; D´=1, r^2=0.068 in Japanese; D´=1, r^2=0.132 in Europeans; D´=0.884, r^2=0.202 in Mexicans).

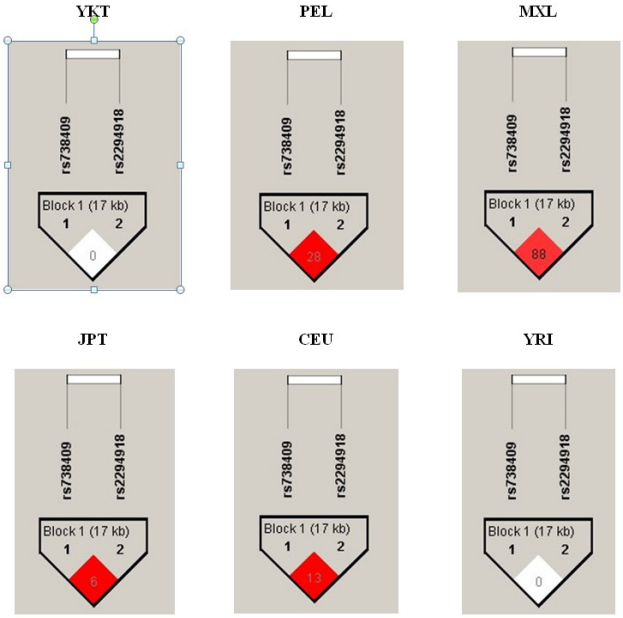

Fig. 1. *LD between the PNPLA3 SNPs*

The color of the cell indicates the adhesion strength between SNPs: red - strong link (D '= 1, LOD> 2), white - weak link (D'<1, LOD <2).

Abbreviations: YKT, Yakuts from Yakutia, Russia; PEL, Peruvians from Lima, Peru; MXL, Mexican Ancestry from Los Angeles, USA; JPT, Japanese in Tokyo, Japan; CEU, Utah Residents (CEPH) with Northern and Western European Ancestry; YRI, Yoruba in Ibadan, Nigeria

Analysis of the distribution of the frequency of genotypes in the studied sample of Yakuts showed the predominance of the carriage of the *PNPLA3* rs738409 GG genotype (57.3%). The *PNPLA3* (rs2294918) AA and AG genotypes carrying the protective A allele are more common in European populations (11.1% and 42.4%, respectively) (Table 3).

Table 3.

Distribution of the PNPLA3 rs2294918 and rs738409 genotypes in Yakuts and in the populations from the "1000 Genomes" project

SNP	Geno-type	Genotype frequency					
		YKT n=150	PEL n=85	MXL n=64	JPT n=104	CEU n=99	YRI n=108
rs738409	GG	0.573	0.529	0.344	0.231	0.040	0.009
	GC	0.293	0.376	0.422	0.385	0.354	0.213
	CC	0.133	0.094	0.234	0.385	0.606	0.778
rs2294918	GG	0.807	0.824	0.672	0.846	0.465	0.824
	GA	0.173	0.153	0.313	0.135	0.424	0.157
	AA	0.020	0.024	0.016	0.019	0.111	0.019

Abbreviations: see Fig.1

The frequency distribution of the *PNPLA3* gene haplotypes for two SNPs (rs738409, rs2294918) based on all detected variants is presented in Table 4. We identified two main haplotypes, the frequency of which was>0.1. One of the most common haplotypes carries variant G (148M), the other carries variant C (148I), and both carry the same variant G (434E). In other words, the more common two haplotypes carry the G (434E) allele, while the A (434K) protective allele does not occur in those found in the two main haplotypes. Protective allele A (434K) is shared by both rare haplotypes. Haplotype G-A (148M-434K) was not found in samples of Peruvians, Japanese, Europeans, and the Yoruba tribe. This haplotype (G-A) was found in Yakuts and Mexicans (6.9% and 1.1%, respectively).

Table 4.

Frequency of I148M-E434K haplotypes in the Yakut population and in the populations from the «1000 Genomes» project

№	Haplotype rs738409-rs2294918	Protein	Haplotype frequency					
			YKT n=150	PEL n=85	MXL n=64	JPT n=104	CEU n=99	YRI n=108
1	G-G	148M-434E	0.651	0.718	0.544	0.495	0.217	0.110
2	C-G	148I-434E	0.243	0.182	0.285	0.495	0.460	0.793
3	G-A	148M-434K	0.069	0	0.011	0	0	0
4	C-A	148I-434K	0.037	0.100	0.161	0.087	0.323	0.091

Abbreviations: see Fig.1

Table 5.

Distribution of diplotypes of two SNP markers of the PNPLA3 gene in the Yakut population and in the populations from the «1000 Genomes» project

№	Genotype / SNP		Diplotype	Diplotype frequency					
	rs738409	rs2294918		YKT (n=150)	PEL (n=85)	MXL (n=64)	JPT (n=104)	CEU (n=99)	YRI (n=108)
1	GG	GG	[GG][GG]	0.453	0.529	0.328	0.221	0.040	0.009
2	CG	GG	[CG][GG]	0.280	0.247	0.234	0.327	0.182	0.176
3	GG	AG	[GG][AG]	0.107	0	0.016	0	0	0
4	CG	AG	[CG][AG]	0.013	0.129	0.188	0.067	0.172	0.037
5	GG	AA	[GG][AA]	0.013	0	0	0	0	0
6	CC	AA	[CC][AA]	0.007	0.024	0.016	0.019	0.111	0.019
7	CC	AG	[CC][AG]	0.053	0.024	0.109	0.067	0.253	0.120
8	CC	GG	[CC][GG]	0.073	0.047	0.109	0.298	0.242	0.639
9	CG	AA	[CG][AA]	0	0	0	0	0	0

Abbreviations: see Fig.1

The distribution of diplotype frequencies for two SNPs (rs738409 and rs2294918) of the *PNPLA3* gene based on all detected variants is presented in Table 5. Eight diplotypes out of nine possible variants were found. The Yakuts often have two diplotypes [GG]-[GG] and [CG]-[GG]. Both diplotypes carry the *PNPLA3* rs738409 G allele (45.3% and 28%) and do not carry the *PNPLA3* rs2294918 A allele. The same distribution of diplotype frequencies is observed among Peruvians (52.9% and 24.7%), Mexicans (32.8% and 23.4%) and Japanese (22.1% and 32.7%). Diplotypes carrying the A protective allele are found at a low frequency (Table 4). The [GG]-[AA] and [CG]-[AA] diplotypes are absent in the population samples, with the exception of the [GG]-[AA] diplotype found in the Yakuts (1.3%). Among the seven discovered diplotypes among the Yoruba tribe, [CC]-[GG] is most common (63.9%). This diplotype does not carry the pathological allele G (rs738409) and does not carry the protective A allele (rs2294918).

Thus, the high frequency of the [GG]-[GG] and [CG]-[GG] diplotypes in Yakuts (45.3% and 25%, respectively), carrying mutant alleles G (rs738409) and not carrying the A allele (rs2294918), indicates that these diplotypes were probably adaptively favorable to the Yakuts. The high frequency of diplotypes [GG]-[GG] and [CG]-[GG] among the indigenous population of America may be due to the settling of the American continent through the Bering Strait by the ancestors of the American Indians.

In conclusion, the analysis of haplotypes and diplotypes based on the markers rs738409 and rs2294918 of the *PNPLA3* gene may contribute to future new biomarkers for the diagnosis of NAFLD and NASH, as well as provide fundamental knowledge on human adaptation to cold.

Competing Interests

The authors declare that they have no competing interests.

Sources of Funding

The study was carried out within the framework of the project "The study of the genetic structure and burden of hereditary pathology of populations of the Republic of Sakha (Yakutia), using the Unique scientific equipment (USE) "The Genome of Yakutia" (Reg. No. USU_507512).

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ORIGINAL ARTICLE

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PNPLA3 rs738409 polymorphism in Patients with Type 2 Diabetes and Concomitant Liver Pathology in Yakutia

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Abstract

Background: The pathogenetic mechanisms of type 2 diabetes (T2D) and non-alcoholic fatty liver disease are closely related. Currently, multiple studies have demonstrated a link between the *PNPLA3* 148M variant and the development and progression of NAFLD, including liver fibrosis. The aim of our research was to study the distribution of alleles and genotypes of the *PNPLA3* rs738409 SNP in Russians and Yakuts living in Yakutia, as well as to search for associations of the *PNPLA3* rs738409 SNP in patients with T2D and non-alcoholic fatty liver disease / non-alcoholic steatohepatitis.

Methods and Results: The study included 179 patients (28 Russians and 151 Yakuts) with T2D and concomitant liver diseases of non-infectious origin. The comparison group consisted of 147 healthy volunteers of Russian ethnicity and 246 healthy volunteers of Yakut ethnicity. The *PNPLA3* 738409 SNP was analyzed by PCR-RFLP reaction. The results found a significant difference between the frequencies of the *PNPLA3* rs738409 genotypes and alleles in Russians and Yakuts, both among healthy volunteers and in T2D patients with liver diseases. The frequency of the G allele occurrence in the group of healthy Yakuts was significantly higher (OR- 3.313; 95% CI: 2.444-4.499; $P<0.001$) than in the group of healthy Russians. No significant differences were found for the *PNPLA3* rs738409 genotype and allele frequencies among a healthy sample and a sample of T2D patients with non-alcoholic fatty liver disease / non-alcoholic steatohepatitis, both in the Russian and Yakut populations. (**International Journal of Biomedicine. 2020;10(4):438-441.**)

Key Words: Type 2 diabetes • non-alcoholic fatty liver disease • *PNPLA3* • rs738409 • I148M

Abbreviations

NAFLD, non-alcoholic fatty liver disease; **NASH**, non-alcoholic steatohepatitis; **PNPLA3**, patatin-like phospholipase domain-containing protein 3; **PCR**, polymerase chain reaction; **RFLP**, restriction fragment length polymorphism; **SNP**, single nucleotide polymorphism; **T2D**, type 2 diabetes.

Introduction

Type 2 diabetes (T2D) is one of the most socially significant pathologies in the Republic of Sakha (Yakutia). T2D often coexists with non-alcoholic fatty liver disease (NAFLD),[(1)] which is considered the most common cause of chronic liver disease.[(2)] The prevalence of NAFLD among patients with T2DM is 60%–80%, and the incidence of NASH is 12%–40%.[(3,4)] Since NAFLD is closely linked to metabolic syndrome, it is frequently recognized as the hepatic manifestation of the metabolic syndrome[(5)] and constitutes the most frequent liver condition worldwide.[(6,7)] The pathogenetic mechanisms of NAFLD and T2D are closely related. In combination, these diseases aggravate each other, significantly, increasing the likelihood of the patient developing liver fibrosis. Whether NAFLD is a consequence or cause of metabolic dysfunction is currently unknown. Early liver diseases occur with unexpressed symptoms or are asymptomatic; therefore, timely diagnosis and prevention of liver diseases, especially

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in patients with T2D, is necessary. The study of the molecular genetic mechanism of the pathogenesis of NAFLD will help in the search for new biomarkers of the disease.[4]

PNPLA3, also known as adiponutrin, which is found in hepatocytes and adipocytes, is one of the candidates potentially related to NAFLD susceptibility.[8,9] PNPLA3 hydrolyses triglycerides and retinyl esters[10] and is associated with NAFLD in GWAS.[11] In 2008, Romeo et al.[11] reported that an allele in PNPLA3 (rs738409[G], encoding I148M) was strongly associated with increased hepatic fat levels (P=5.9×10^{-10}) and with hepatic inflammation (P=3.7×10^{-4}). Currently, multiple studies have demonstrated a link between the *PNPLA3* 148M variant and the development and progression of NAFLD, including liver fibrosis.[12-17]

The aim of our research was to study the distribution of alleles and genotypes of the *PNPLA3* rs738409 SNP in Russians and Yakuts living in Yakutia, as well as to search for associations of the *PNPLA3* rs738409 SNP in patients with T2D and NAFLD/NASH.

Materials and Methods

The study of the *PNPLA3* SNPs (rs2294918 and rs738409) was carried out in the Department of Molecular Genetics at YSC CMP. For the study, we used DNA samples from the collection of biomaterials of the YSC CMP (Project "The Genome of Yakutia"; No. USE_507512). The study included 179 patients (28 Russians and 151 Yakuts) with T2D and concomitant liver diseases of non-infectious origin. Exclusion criteria: autoimmune hepatitis, primary biliary cholangitis, primary sclerosing cholangitis, hereditary hemochromatosis, Wilson-Konovalov disease, and alcohol abuse (>30 g/l). The comparison group consisted of 147 healthy volunteers of Russian ethnicity and 246 healthy volunteers of Yakut ethnicity. The biomaterial was collected during expeditions conducted in the Central regions of Yakutia. Ethnicity was taken into account up to the third generation.

Genomic DNA was isolated using the standard phenol-chloroform extraction method from frozen whole blood. After DNA extraction, the samples were subjected to a PCR-RFLP reaction to analyze the *PNPLA3* rs738409 SNP.

The conditions for the amplification of the region of the gene containing the polymorphic variant, indicating the sequence of oligonucleotide primers, the restriction enzyme used and the lengths of the restoration fragments, are presented in Table 1.

Table 1.

Conditions for PCR-RFLP analysis

Primer	Annealing temperature	Length of amplicate, bp	Restriction enzyme	Restriction fragment length, bp
F: 5'-TGGGCCTGA AGTCCGAGGGT-3'	66 °C	333 bp	BstF5 I	CC – 200, 133 CG – 333, 200, 133 GG – 333
R: 5'- CCGACACCA GTGCCCTGCAG-3'				

bp- base pairs

Genotypes were determined by analyzing the sizes of the resulting fragments by gel electrophoresis on 4% agarose gel with ethidium bromide in standard Tris-acetate buffer at 120V for 1 hour. Restriction products were visualized using a gel documentation system in a Vilber Lourmat Compact UV Transilluminator (France) (Fig.1).

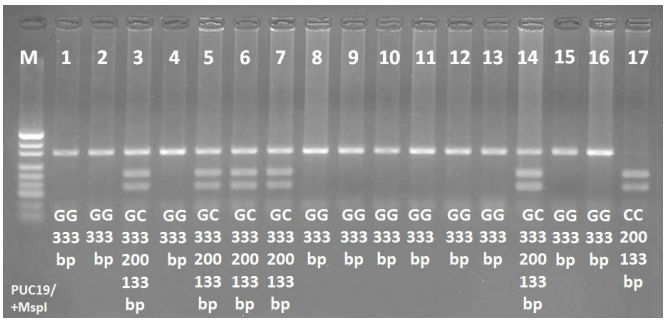

Fig. 1. *PCR-RFLP analysis of PNPLA3 gene (rs738409) C>G genotypes with restriction endonuclease enzyme BstF5 I.*

The study was approved by the Ethics Committee of the Yakut Science Center of Complex Medical Problems (YSC CMP). Written informed consent was obtained from each research participant (or the participant's parent/guardian).

Statistical analysis was performed using the Statistica 8.0 software package (Stat-Soft Inc., USA). The correspondence of the distributions of genotypes to the expected values at HWE and comparison of the frequencies of allelic variants/genotypes were performed using the chi-square test. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated. A probability value of $P<0.05$ was considered statistically significant.

Results and Discussion

An analysis of the frequency distribution of alleles and genotypes of the *PNPLA3* rs738409 SNP in the studied samples showed a difference between Yakuts and Russians, both in healthy volunteers and among those suffering from liver diseases (Table 2).

Table 2.

The frequency distribution of alleles and genotypes of the PNPLA3 rs738409 SNP in Russians and Yakuts

Samples	n	Division	Genotype frequency, %			Allele frequency		χ^2	P
			CC	CG	GG	C	G		
Population sample of the Yakuts	246	O	13.8	26.4	59.8	0.270	0.730	26.825	0.000
		E	7.3	39.4	53.2				
Population sample of Russians	147	O	41.5	27.2	31.3	0.551	0.449	29.77	0.00
		E	30.4	49.5	20.2				
A sample of Yakut patients	151	O	9.9	29.8	60.3	0.248	0.752	6.147	0.013
		E	6.2	37.3	56.5				
A sample of Russian patients	28	O	35.7	25.0	39.3	0.482	0.518	6.982	0.008
		E	23.2	49.9	26.8				

O – Observed; E – Expected

The sample of healthy patients of Russian ethnicity had the largest number of carriers of the homozygous CC genotype, while the samples of patients with T2D were dominated by carriers of the homozygous GG genotype. Among patients with T2D and a sample of healthy Yakut ethnicity, the largest number was carriers of the GG genotype.

We found significant differences between the frequencies of the *PNPLA3* rs738409 genotypes and alleles in healthy volunteers of Yakut and Russian ethnic groups (Table 3). The frequency of the C allele and the CC homozygous genotype prevailed in the sample of healthy volunteers of Russian ethnicity, while the frequency of the G allele and the homozygous GG genotype prevailed in the sample of healthy volunteers of Yakut ethnicity. The calculation of the odds ratio showed that the frequency of occurrence of the G allele in the Yakut group was significantly higher (OR=3.313; 95% CI: 2.444-4.499; $P<0.001$) than in the sample of Russian ethnicity.

Table 3.

The frequency distribution of alleles and genotypes of the PNPLA3 rs738409 SNP among healthy volunteers of Yakut and Russian ethnic groups

Genotype/ allele	Yakuts (n=246)	Russians (n=147)	χ^2	OR (95% CI)	*P*-value
C/C	34 (13.8%)	61 (41.5 %)	44.357	3.313 (2.444-4.499)	0.000*
C/G	65 (26.4%)	40 (27.2 %)			
G/G	147 (59.8%)	46 (31.3 %)			
C	0.270	0.551	60.653		0.000**
G	0.730	0.449			

P- the achieved level of significance in comparing the distribution of genotypes (*) and allele frequency (**)

We found no significant differences in the frequency distribution of the *PNPLA3* rs738409 genotypes and alleles among healthy Russians and Russian T2D patients with concomitant liver diseases (Table 4). In the sample of T2D patients of Russian ethnicity with concomitant liver pathologies, the homozygous GG genotype prevailed, which is probably due to the small number of samples (n=28). The homozygous CC genotype prevailed among the sample of healthy volunteers of Russian ethnicity.

We found no significant differences in the frequency distribution of genotypes and alleles among healthy Yakuts and Yakut T2D patients with concomitant liver diseases (Table 5). According to the open-source data of the 1000 Genomes project,[18] the *PNPLA3* rs2294918 G allele is found with a high frequency in populations of Central and South America (Peruvians - 71.8%, Mexicans - 55.5%, Colombians – 41%). Attention is drawn to the fact that the higher the frequency of the G allele, the higher the percentage of indigenous people in the population. For example, in the population of Puerto Rico, where the Native American population is 11%, the frequency of the G allele is 31.7%. In Europeans, the frequency of the G allele is on average 22.6%. Among Asians, the Japanese have a high frequency of the G allele (42.3%). The owners of the lowest frequency of the G allele are Africans (8.6%-17.2%).

Table 4.

The frequency distribution of alleles and genotypes of the PNPLA3 rs738409 SNP among healthy Russians and Russian T2D patients with concomitant liver diseases

Genotype / allele	Patients (n=28)	Control sample (n=147)	χ^2	OR (95% CI)	*P*-value
C/C	10 (35.7 %)	61 (41.5 %)	0.698	1.318 (0.744-2.336)	0.705*
C/G	7 (25 %)	40 (27.2 %)			
G/G	11 (39.3 %)	46 (31.3 %)			
C	0.482	0.551	0.643		0.423**
G	0.518	0.449			

P–the achieved level of significance in comparing the distribution of genotypes (*) and allele frequency (**)

Table 5.

The frequency distribution of alleles and genotypes of the PNPLA3 rs738409 SNP among healthy Yakuts and Yakut T2D patients with concomitant liver diseases

Genotype / allele	Patients (n=151)	Control sample (n=246)	χ^2	OR (95% CI)	*P*-value
C/C	15 (9.9)	34 (13.8)	1.535	1.121 (0.808-1.557)	0.464*
C/G	45 (29.8)	65 (26.4)			
G/G	91 (60.3)	147 (59.8)			
C	0.248	0.270	0.361		0.548**
G	0.752	0.730			

P – the achieved level of significance in comparing the distribution of genotypes (*) and allele frequency (**)

In conclusion, our study found a significant difference between the frequencies of the *PNPLA3* rs2294918 genotypes and alleles in Russians and Yakuts, both among healthy volunteers and in T2D patients with liver diseases. The frequency of the G allele occurrence in the group of healthy Yakuts was significantly higher (OR=3.313; 95% CI: 2.444-4.499; $P<0.001$) than in the group of healthy Russians. No significant differences were found for the *PNPLA3* rs2294918 genotype and allele frequencies among a healthy sample and a sample of T2D patients with NAFLD/NASH, both in the Russian and Yakut populations.

Competing Interests

The authors declare that they have no competing interests.

Sources of Funding

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ORIGINAL ARTICLE

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Association between Health-Related Quality of Life and Emotional Problems in Rural Adolescents with Overweight and Obesity

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Abstract

Background: Health-related quality of life (HRQL) and emotional functioning are important parameters of weight-loss motivation. The aim of this study was to identify the association between emotional/personal characteristics and HRQL in adolescents with overweight and obesity.

Methods and Results: Our cross-sectional case-control study included 172 adolescents: 19 of them overweight, 67 obese, and 86 healthy. We measured anthropometry and performed a psychological examination (PedsQL 4.0, The State-Trait Anxiety Inventory, Buss-Durkee Hostility Inventory, and Personality inventory Mini-SMIL). Negative correlations prevailed between PedsQL scales and psychological traits of healthy adolescents: increases in anxiety, covert anger, and depression were associated with a decrease of HRQL (Total Scale Scores, Psychosocial Health Score, Physical Health Score, and Emotional, Social and School functioning). The psychological impairment (Irritability, Resentment, Guilt, Covert anger) positively correlated with a change in HRQL (Total Scale Scores, Psychosocial Health Score, and Physical Health Score) in obese adolescents.

Conclusion: Obese adolescents had an inadequate association between HRQL and emotional state: the accumulation of anxiety and aggression was accompanied by an increase in HRQL. These difficulties can be a barrier to having treatment and weight-loss motivation.(**International Journal of Biomedicine. 2020;10(4):442-447.**)

Key Words: anxiety • anger • emotion • weight-loss motivation

Abbreviations

BW, body weight; **BMI**, body mass index; **HRQL**, health-related quality of life

Introduction

Childhood obesity is one of the most prevalent health problems; it is associated with a high risk of obesity outcomes,[1] including a decrease in psychological well-being. It has been established that there is an association between children's overweight/obesity and some mental health problems, including anxiety,[2-4] aggression and depression,[5,6] dissatisfaction with the body,[7,8] low self-esteem,[9] and some behavioral problems.[10] Moreover, previously we have found that obesity in adolescents is associated with decreased HRQL scores.[11]

HRQL is an important parameter of well-being, because being obese creates psychological stress for children. It has been established that severely obese children and adolescents have lower HRQL than children and adolescents who are healthy and that they have a quality of life similar to those diagnosed with cancer.[12] Usually, a specific level of HRQL is described in groups of obese and healthy children.[13] A number of studies have shown the relationship between emotional disorders and HRQL in adult obese patients,[14] but we have not found data about a relationship between emotional traits and HRQL in rural adolescents with overweight and obesity.

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Because an emotional eating style can contribute to psychological problems and can play a considerable role in increased appetite,[15] it is important to know the emotional status of adolescents with overweight and obesity. We suggest that adequate perception of weight, well-being, and emotional functioning determines the motivation to lose weight. We do not exclude that obesity contributes to psychological problems, but in this study, we consider a model of externalizing behavioral problems, which indicates that emotional and personal problems might foster weight gain.[16]

Thus, the hypothesis for our study: Emotional status is associated with the HRQL in overweight and obese adolescents; namely, emotional problems may deform the perception of well-being and illness, hence reducing the motivation to lose weight. In this regard, the aim of this study was to identify the association between emotional/personal characteristics and HRQL in adolescents with overweight and obesity.

Materials and Methods

Study design

We conducted a cross-sectional case-control study of adolescents living in rural areas of the Republic of Buryatia (RB) within an annual medical examination. A total of 1,456 adolescents (11-17 years) were surveyed by the continuous sampling method. Each adolescent was given an anthropometric examination that included linear growth, height, BW, BMI. According to the results of the examination, overweight and obesity were diagnosed in 86 adolescents. To achieve the purpose of the study, a control group of adolescents with normal weight was formed from the same population sample, comparable in age and sex (n=86). Thus, the analyses included a total of 172 subjects.

Criteria for inclusion in the study were: 1) subjects aged between 11 and 17 years; 2) voluntary informed consent of adolescents over 15 years old, parents/legal representatives of adolescents to participate in the study. Exclusion criteria for the study were: 1) weight deficit (BMI z-scores <5th percentile); 2) patients or their parents unable to provide the requested information. The study was performed in the territory of 9 out of 15 municipal areas of the RB. Participants were recruited from all adolescents (continuous sampling) who had passed the annual scheduled medical examination.

Procedures and measures

This study included measurement of such anthropometric parameters as linear growth, height, BW, BMI. BW was considered excessive with a BMI>85th percentile of distribution for a given sex and age, obesity was established with BMI>95th percentile. BMI values were reformed using BMI z-scores. Height and weight parameters of participants were evaluated using reference values of the WHO AnthroPlus calculator.

Psychological assessment of the protocol research was performed in a group format in the classroom. Each adolescent was asked to complete the following questionnaires:

1.The State-Trait Anxiety Inventory (STAI) – the Russian version, adapted by Khanin.[17] The Trait and State scales measure trait and situational anxiety, respectively.

2. Buss-Durkee Hostility Inventory (BDHI) – the Russian version adapted and standardized by Khvan et al.[18] The BDHI is a 75-item, self-report inventory intended to measure aspects of anger/hostility and aggression. The BDHI comprises subscales labeled Assault, Indirect, Irritability, Negativism, Resentment, Suspicion, Guilt, and Verbal aggression. As a result, two factors are analyzed: Covert anger/Neurotic aspects of hostility (Suspicion+Resentment) and Overt anger/Behavioral-expressive aspects of hostility (Indirect+Assault+Verbal).

3. Personality inventory Mini-SMIL consists of 65 questions and assesses different personality traits.[19] The Mini-SMIL is made up 10 subscales, which are a result of answering certain questions on the test: Scale 1 (Hypochondria), Scale 2 (Depressive symptoms), Scale 3 (Emotional and vegetative instability), Scale 4 (Ambitiousness and irritability), Scale 5 (Masculinity-Femininity), Scale 6 (Stubbornness: suspiciousness, paranoia), Scale 7 (Anxiety), Scale 8 (Odd thinking, social alienation), Scale 9 (Level of excitability, hypomania), and Scale 0 (Introversion).

4. HRQL was measured by using the validated Pediatric Quality of Life Inventory 4.0 Generic Core Scales (PedsQL).[20] The PedsQL is a 23-item age-specific survey. This questionnaire assesses physical, emotional, social, and school functioning, from which total, physical, and psychosocial health summary scores are derived.

The study was carried out in compliance with Ethical Principles for Medical Research Involving Human Subjects, Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964, and amended by the 64th WMA General Assembly, Fortaleza, Brazil, October 2013. The study was approved by the Ethics Committee of the Scientific Center for Family Health and Human Reproduction Problems. Written informed consent was obtained from each patient.

Statistical analysis was performed using STATISTICA 6.1 software (Stat-Soft Inc., USA). The normality of distribution of continuous variables was tested by one-sample Kolmogorov-Smirnov test. 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. The frequencies of categorical variables were compared using Pearson $\chi 2$ Spearman's rank correlation coefficient 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

Table 1 displays the demographic and anthropometric data of adolescents with normal BW, overweight, and obesity. There were significant differences between adolescents with normal BW, overweight, and obesity with regard to weight, BMI, and z-scores BMI. All these indicators were most high in adolescents with obesity.

Table 2 shows the mean scores on the scales of psychological questionnaires for adolescents. Only two scales of personality inventory Mini-SMIL were significantly

different: the Scale 5 score was less in obese adolescents than in adolescents with normal BW. The Scale 0 score was lower in a group of overweight adolescents than in healthy adolescents. The scores of other scales and subscales did not significantly differ.

Table 1.

Demographic and clinical features for adolescents with normal BW, overweight, and obesity

Variable	Normal BW (1)	Overweight (2)	Obesity (3)	*P*-value
Age, yrs	14.11±1.78	13.73±2.23	13.89±1.85	0.6321
Boys, n (%)	48 (55.56)	10 (52.63)	46 (68.66)	0.150
Height, cm	161.55±9.77	162.87±9.05	163.1±12.27	0.6566
Weight, kg	52.33±9.71	62.92±11.93	78.97±15.86	P=0.0000 P_{1-2}=0.0034 P^{1-3}=0.0000 P_{2-3}=0.0000
BMI, kg/m^2	19.91±2.47	23.71±3.05	29.42±3.67	P=0.0000 P_{1-2}=0.0000 P^{1-3}=0.0000 P_{2-3}=0.0000
z-scores BMI,	0.07±0.64	1.50±0.36	2.51±0.55	P=0.0000 P_{1-2}=0.0000 P^{1-3}=0.0000 P_{2-3}=0.0000

Table 3 demonstrates HRQL by PedsQL for adolescents. After assessing the differences between the 3 groups, we carried out a pairwise comparison. There were no significant differences in the level of PedsQL scales between adolescents with overweight and normal BW and obesity. The scores of Total HRQL and other subscales, except for Emotional functioning, were significantly lower for obese adolescents than for adolescents with normal BW.

Correlations between HRQL and psychological features in adolescents with different weight category

Spearman correlations between PedsQL scores and psychological parameters of the normal weight, overweight and obese adolescents are reported in Table 4. Opposite tendencies of these correlations were identified in groups with different BW.

Negative significant correlations prevailed between PedsQL scales and psychological features in adolescents with normal BW. For example, all summary scales, such as Total Scale Scores, Psychosocial Health Score, and Physical Health Score are associated with BDHI-Resentment, BDHI-Suspicion, BDHI-Covert anger, STAI-state subscale, and STAI-trait subscale. Besides, these PedsQL Summary Scales are correlated with such Mini-SMIL scales as Scale 2, Scale 4, Scale 6, Scale 7, Scale 8, and Scale 0. Thus, an increase in anxiety, covert anger, resentment, depressive symptoms, introversion, and other features are associated with a decrease in the subscales of HRQL (Total Scale Scores, Psychosocial Health Score, Physical Health Score, and Emotional, Social and School functioning) in adolescents with normal BW.

The STAI-trait subscale is significantly correlated (inverse association) with the Physical Health Score (r=-0.714). An increase in BDHI (Negativism) is associated with an increase in Psychosocial Health Score (r=0.501). The Mini-SMIL Scale 9 is protective in relation to Physical Health Score. In this way, the direction of correlation in overweight patients is changing. These changes are not very pronounced, but are statistically significant.

Table 2.

Scores on psychological measures for adolescents with normal BW, overweight, and obesity

Psychological subscales	Normal BW (1)	Overweight (2)	Obesity (3)	*P*- value
STAI-state subscale	38.68±9.93	36±7.52	39±1.41	0.2961
STAI-trait subscale	40.88±10.54	41.86±7.95	41.5±13.44	0.9174
BDHI (Assault)	4.75±2.19	4.61±1.88	4.94±2	0.7768
BDHI (Indirect)	3.68±1.62	3.56±1.98	3.87±1.53	0.6791
BDHI (Irritability)	4.71±2.27	4.28±2.49	4.28±2.22	0.4656
BDHI (Negativism)	2.35±1.3	2.47±1.12	2.6±1.35	0.5006
BDHI (Resentment)	3.7±2	3.78±1.35	3.7±1.66	0.9837
BDHI (Suspicion)	4.7±1.83	5.06±2.33	4.85±1.51	0.6937
BDHI (Guilt)	5.8±2.08	6.44±1.42	6±2.13	0.4508
BDHI (Verbal aggression)	5.89±2.43	6.67±2.05	6.02±2.52	0.4496
BDHI (Covert anger)	8.33±3.25	8.83±2.9	8.22±2.5	0.7266
BDHI (Over anger)	15.19±5.25	15.56±3.82	15.01±5.48	0.9184
Mini-SMIL Scale 1	55.74±10.78	58.33±11.93	55.85±6.7	0.5491
Mini-SMIL Scale 2	54.26±10.78	51.67±11.93	51.71±9.46	0.2761
Mini-SMIL Scale 3	57.96±9.84	59.17±9	55.85±9.48	0.2690
Mini-SMIL Scale 4	65.19±9.9	62.5±12.15	64.63±9.77	0.5771
Mini-SMIL Scale 5	60.76±9.87	55±13.82	55.37±9.51	P=0.0026 P_{1-2}=0.0705 P^{1-3}=0.0042 P_{2-3}=0.9894
Mini-SMIL Scale 6	60.69±13.9	62.5±14.85	61.46±12.95	0.8536
Mini-SMIL Scale 7	58.33±12.93	53.33±11.55	58.53±13.52	0.2770
Mini-SMIL Scale 8	62.22±14.75	55±14.46	60.24±13.71	0.1355
Mini-SMIL Scale 9	75.46±12.78	78.33±10.3	79.27±11.49	0.1428
Mini-SMIL Scale 0	59.07±14.37	48.18±8.74	53.29±13.58	P=0.0017 P_{1-2}=0.0052 P^{1-3}=0.0262 P_{2-3}=0.3181

Table 3.

Total and Subscales Scores PedsQL for adolescents with normal BW and obesity

PedsQL subscales	Normal BW (n=86)	Obesity (n=67)	*P*-value
Physical health score	86.31±13.93	80.6±17.38	0.0255
Psychosocial Health Score	78.43±13.03	72.54±13.79	0.0076
Emotional functioning	72.66±17.64	68.43±17.52	0.1420
Social functioning	87.55±13.71	80.52±15.91	0.0039
School functioning	75.06±17.14	68.66±16.27	0.0205
Total Scale Score	81.18±11.95	75.34±14.04	0.0062

Table 4.

Spearman's correlation coefficients between PedsQL scores and BDHI, STAI, Mini-SMIL scores

	Total Scale Score	PhH score	PsH Score	EF	SoF	SchF
Adolescents with normal BW						
BDHI (Assault)	0.151	-0.14	0.127	0.098	0.180*	0.046
BDHI (Irritability)	-0.212*	-0.208*	-0.162	-0.225*	-0.016	-0.13
BDHI (Resentment)	-0.247*	-0.254^	-0.192*	-0.249*	-0.182*	-0.09
BDHI (Suspicion)	-0.247*	-0.177*	-0.22*	-0.224*	-0.145	-0.214*
BDHI (Guilt)	-0.213*	-0.15	-0.193*	-0.273^	-0.126	-0.109
BDHI (Covert anger)	-0.296^	-0.242*	-0.263^	-0.304^	-0.178*	-0.189*
STAI-state subscale	-0.431^	-0.302^	-0.459^	-0.447^	-0.35^	-0.304*
STAI-trait subscale	-0.414^	-0.276^	-0.446^	-0.428^	-0.342^	-0.336^
Mini-SMIL, Scale 1	-0.22*	-0.247*	-0.156	-0.212*	-0.207*	0.025
Mini-SMIL, Scale 2	-0.363^	-0.31^	-0.354^	-0.326^	-0.272^	-0.288^
Mini-SMIL, Scale 4	-0.258*	-0.224*	-0.211*	-0.191	-0.062	-0.259*
Mini-SMIL, Scale 6	-0.221*	-0.216*	-0.21*	-0.206*	-0.077	-0.166
Mini-SMIL, Scale 7	-0.342^	-0.27*	-0.321^	-0.341^	-0.23*	-0.173
Mini-SMIL, Scale 8	-0.302^	-0.2*	-0.289^	-0.278^	-0.188	-0.184
Mini-SMIL, Scale 0	-0.276^	-0.295^	-0.241*	-0.255*	-0.238*	-0.105
Adolescents with overweight						
BDHI (Negativism)	0.444	0.462	0.501*	0.546*	0.342	0.267
STAI-trait subscale	-0.605	-0.714*	-0.236	-0.271	-0.676	-0.333
Mini-SMIL, Scale 9	0.406	0.588*	0.335	0.297	0.468	0.436
Adolescents with obesity						
BDHI (Irritability)	0.331*	0.064	0.35*	-0.092	0.424^	0.125
BDHI (Resentment)	0.36*	0.332*	0.369*	0.251	0.371*	0.126
BDHI (Guilt)	0.355*	0.35*	0.392*	0.221	0.432^	0.228
BDHI (Covert anger)	0.357*	0.326*	0.384*	0.212	0.375*	0.274
Mini-SMIL, Scale 9	-0.231	-0.178	-0.311*	-0.129	-0.301	-0.287

PhH, Physical Health; PsH, Psychosocial Health; EF, Emotional Functioning; SoF, Social Functioning; SchF, School Functioning;

**- Spearman's correlation P<0.05; ^- Spearman's correlation P<0.005.*

The changes in psychological functioning positively and significantly correlate with change in HRQL in adolescents with obesity. For example, Total Scale Scores, Psychosocial Health Score, Physical Health Score are associated with BDHI (Resentment), BDHI (Guilt), BDHI (Covert anger). Besides, a negative significant correlation has been found between Psychosocial Health Score and the Mini-SMIL Scale 9. Thus, we detected the opposite direction of associations between psychological features and HRQL in adolescents with obesity, compared with adolescents with normal BW.

In the first stage of this study, we found that obese adolescents differ from healthy peers in the way their interact with society: They are more masculine and aggressive. Moreover, obese adolescents are more extroverted than overweight adolescents. Our results are in good agreement with the results of other researchers who identified that extraversion is associated with obesity in children.[21] Behavioral differences between introverts and extroverts are associated with self-regulation of excitation.[22] Particularly, more introverted individuals tend to control and reduce excitation levels, while

extroverts can further increase their arousal level through food intake.[23] The main idea in the concept of self-regulation is the ability to distinguish impulses, as well as to control behavior in a negative situation, and to follow rules or suppress immediate desires, and finally to delay gratification.[24,25] In this way, psychological and emotional disorders and obesity have a common genetic basis, consisting in the regulation of monoamines and peptides, including serotonin, norepinephrine, dopamine, and corticotropin-releasing hormone.[26]

The second objective of this study was to identify HRQL differences in adolescents with different body weight. Our results show that only HRQL in the domain of Emotional functioning did not significantly differ between obese and healthy adolescents. Our data are consistent with the results of a study in Kuwait.[27] However, other studies conducted on urban children have shown a decrease in all domains of HRQL in obese adolescents, in comparison with healthy adolescents.[28,29] According to our results, the HRQL decreases in adolescents with obesity, but not in those who are overweight. Consequently, a large increase in BW significantly reduces HRQL, which is consistent with data found in the literature.[12]

The third, and main, aim of this study was to identify the relationship between psychological features and HRQL in obese, overweight, and healthy adolescents. Negative significant correlations prevailed between PedsQL scales and psychological features in healthy adolescents. An increase in Anxiety, Covert anger, Resentment, Depressive symptoms, and Introversion was associated with a decrease in the subscales of HRQL (Total Scale Scores, Psychosocial Health Score, Physical Health Score, Emotional, Social and School functioning) in healthy adolescents. Comparable results were obtained in a group of overweight adolescents.

Meanwhile, in obese adolescents, the direction of correlation is changing in the opposite direction. The rise in aggression parameters positively correlated with changes in Total Scale Score, Psychosocial Health Score, and Physical Health Score. The level of excitability negatively correlated with Psychosocial Health Score; consequently, the level of excitability can be a protector from psychosocial functioning dissatisfaction. The process of false quality of life assessing occurs through the negative evaluation of one's own emotions and wrong assessment of the behavior and emotions of other people. The direct association between the accumulation of aggressive emotions and the increase in HRQL might be the result of errors in the evaluation and understanding of one's own emotions and failure. Incorrect interpretation of their own emotions does not allow adolescents to understand the psychological causes of eating disorders. This fact can significantly distort the awareness of the psychological causes of increased appetite and reduce the motivation for weight loss.

Several factors increase the strength of our results: the analysis of a general population sample of obese and overweight adolescents living in rural areas of the RB; the use of well-validated psychological questionnaires; the high participation rate, which minimizes sample selection bias. However, there are several limitations of this study, which should be noted. First, we analyzed a non-clinical sample of healthy, overweight, and obese children, except for a clinical sample of severely obese patients seeking treatment in a specific department. A sample of severely obese patients has more complicated pathologies, including greater psychological distress and more eating disorders. Further research is needed to examine all these categories of adolescents with different BW in order to identify the dynamics of HRQL, psychological and psychopathological changes, and their interactions depending on extreme weight gain in adolescents. Second, we have not analyzed the parents' version of PedsQL, which is filled out by the parents. An analysis of the parents' perception of the obese adolescent's quality of life might complete our knowledge about parental attitudes to childhood obesity in a population. Third, the cross-sectional design does not allow for making predictions and it will be important to conduct a longitudinal study of the relationship between emotions and HRQL.

Despite these limitations, our study confirms the close relationship between a psychological statement and HRQL in obese adolescents. Based on the present findings, we suggest a more direct focus on the study of obese adolescent's psychological and emotional characteristics, or a need for understanding the reasons for aggression and coping behavior to improve the emotional well-being, quality of life, and motivation to lose weight. If psychological problems are a barrier to having recommendations and treatment, then psychological interventions should follow.[30]

Summarizing these results, we can conclude that obese and healthy adolescents have opposite directions in the relationship between emotional/personal characteristics and HRQL. In healthy adolescents, the HRQL changes demonstrate an adequate level according to their emotional state. In adolescents with obesity, the accumulation of aggressive emotions is accompanied by an increase in the HRQL. It may be the main reason for weight gain. This fact requires targeted psychological preventive work with overweight and obese adolescents, aimed at treating the psychological causes of increased appetite and the rise of motivation to lose weight.

Competing Interests

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Adolescent Health

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Change in the Motivation to Receive Treatment by Overweight Adolescent Girls in the Course of Counseling

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Abstract

The purpose of this study was to reveal changes in the motivation to receive treatment by overweight adolescent girls in the course of counseling.

Methods and Results: The study included 2 groups of overweight girls with gynecological disorders aged between 14 years and 16 years. Group 1 comprised 20 girls admitted to a hospital for the first time; Group 2 consisted of 20 girls who had undergone treatment earlier (were admitted repeatedly). The motivational-personal sphere was studied using the following methods: (1) McClelland's method for testing social and psychological activity motivators; (2) S.R. Panteleev's research method of self-attitude; (3) EAT-26; (4) The body satisfaction method by Collins.

The results obtained showed that changing motivation to receive treatment is a complex process implying changes in motivational induction: Achievement Motive, Affiliation Motive, Help Motive, Development Motive, and Cognition Motive. It was also revealed that directly acting motives prevailed over those emerging due to awareness of the treatment's purpose.

Conclusion: A specially developed counseling process comprising diagnostic, informational, correctional and developmental aspects of working with adolescent girls individually and in groups contributed to changing motivation to receive treatment by overweight adolescent girls. (**International Journal of Biomedicine. 2020;10(4):448-452.**)

Key Words: overweight • adolescent girls • counseling • motivation • personal sphere

Introduction

Various aspects of being overweight are studied because of the great medical and psychological significance, widespread prevalence, and continuous growth of the number of overweight people in all age groups, including adolescents.[(1)] According to the WHO, 10%-15% of the child population in developed countries is overweight or obese. The results of sample studies in Russia showed a high prevalence of obesity among children over the past 20 years. The number of obese children aged 6-11 years has doubled (from 7% to 13%), and the number of obese adolescents aged 12-19 years has increased almost 3 times (from 5 to 14%), which leads to impaired puberty in adolescents, especially in girls, and to impaired reproductive function in the future.[(2)]

In recent years, more studies have been conducted that demonstrate that the satiety signal triggers complex reactions of the hypothalamus-pituitary and limbic systems, some of which are associated with positive emotions.[(3-6)] An unfavorable emotional state, low self-esteem, the degree of functional defect caused by the disease and subjective perception, as well as the patient's personal reaction to the disease, affect the motives for treatment, expressed in the denial of excess weight and self-acceptance.[(7-9)]

Changes in the motivation to receive treatment by overweight adolescents and a higher effectiveness of adolescents' involvement in the treatment process are possible in the course of specially organized counseling.[(10-14)] The urgency of the problem of changing the treatment motives in overweight adolescents, on the one hand, and its

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underdevelopment, on the other hand, determined the purpose of the study.

The purpose of this study was to reveal changes in the motivation to receive treatment by overweight adolescent girls in the course of counseling.

Materials and Methods

The study included 2 groups of overweight girls with gynecological disorders (primary oligomenorrhea, secondary olegomenorrhea, hypothalamic dysfunction (menstrual irregularities) aged between 14 years and 16 years. Group 1 comprised 20 girls admitted to a hospital for the first time; Group 2 consisted of 20 girls who had undergone treatment earlier (were admitted repeatedly). The data obtained as a result of the adolescents' examinations at the Scientific Center for Family Health and Human Reproduction Problems was collected in the form of a questionnaire survey, accompanied by studying the background, and general clinical and psychological study findings.

The motivational-personal sphere was studied using the following methods: (1) McClelland's method for testing social and psychological activity motivators; (2) S.R. Panteleev's research method of self-attitude; (3) EAT-26; (4) The body satisfaction method by Collins.

The study was approved by the Ethics Committee of the Scientific Center for Family Health and Human Reproduction Problems. Written informed consent was obtained from each research participant (or the participant's parent/guardian).

Statistical analysis was performed using the statistical software package SPSS version 21.0 (IBM Corp., Armonk, N.Y., USA). Baseline characteristics were summarized as frequencies and percentages for categorical variables and as mean±SD for continuous variables. The Wilcoxon criterion was used to compare the differences between the paired samples. A value of $P<0.05$ was considered significant.

Results and Discussion

Study of changing the motivation to receive treatment by overweight adolescents in the course of counseling comprised 6 stages (incoming diagnostics was performed at the second counseling stage, intermediate diagnostics was performed at the fourth counseling stage, and the final diagnostics was performed at the sixth counseling stage).[15]

The first stage of this six-stage program was acquaintance, study of the state. The second stage was a two-dimensional definition of the problems associated with the motives to receive treatment. The third stage was identification of alternatives to the motives to receive treatment by overweight adolescents. The fourth stage was planning, development, and consolidation of behavioral strategies for developing treatment motivation. The fifth stage was development of motives by overweight adolescents outside the counseling process. The sixth stage was assessment and feedback.

Our study of changes in the motivational induction characteristics in the girls of Group 1 revealed the following: Achievement Motive, the most pronounced motivation, was found in 5.67% of cases, Influence Motive in 1.27%, Aggression Motive in 1.13%, and less pronounced Spiritual Motive in <1.0% (Fig.1).

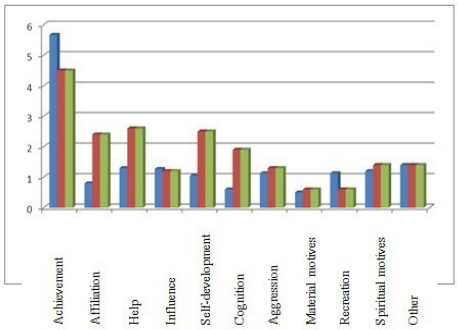

Fig.1. *Changes in motivational induction characteristics in the girls of Group 1.*

– Incoming diagnostics; – Intermediate diagnostics; – Final diagnostics

Obtained intermediate diagnostics results: Using a variety of techniques aimed at reducing motivation, a decrease in Achievement Motive was registered with a simultaneous increase in Affiliation Motive ($P<0.05$), Help Motive ($P<0.05$) and Self-Development Motive ($P<0.05$). The patients became more focused on pro-social motives. They were encouraged to take part in the treatment by procedural-meaningful motives when they were satisfied with the process and content of the exercises, and not by factors that are not related to therapeutic activity.

The final diagnostics results produced the following data: No significant changes were recorded in the motivation system. The results of Nuttin's test and McCelland's method showed no significant changes. The obtained results showed that, in general, the motives of adolescents stabilized at the second stage of the study and further self-perception changes occurred.

Thus, significant strengthening in the following motivational induction characteristics was observed: Achievement Motive, Affiliation Motive, Help Motive, Development Motive, and Cognition Motive.

The study of changes in the motivational induction characteristics in overweight adolescent girls of Group 2 revealed the following: Achievement Motive, the most pronounced motivation, was found in 5.67% of cases, Influence Motive in 1.27%, Aggression Motive in 1.13%, and less pronounced Spiritual Motive in <1.0%. Achievement Motive is the adolescent's persistent need to be successful in various activities. During the initial diagnosis, low motivation and lack of interest in the treatment process were found (Fig.2).

Obtained intermediate diagnostics results showed no significant differences in the level of motivation in the adolescents of Groups 1 and 2. We registered decreased achievement motivation with simultaneous growth of the affiliation motives using various techniques aimed at strengthening motivation.

The final diagnostics showed the following results. Subjects' motivation stabilized at the second counseling stage

and further self-perception changes occurred. The adolescents became more focused on the treatment and started to take part in psychological exercises.

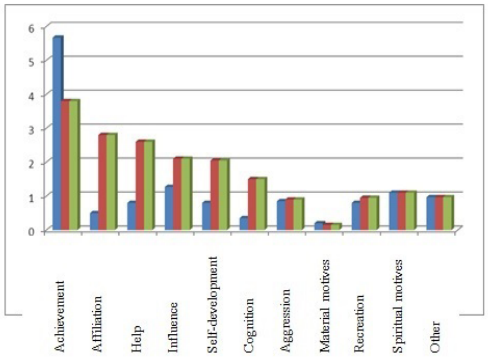

Fig.2. *Changes in motivational induction characteristics in the girls of Group 2.*

– Incoming diagnostics; – Intermediate diagnostics; – Final diagnostics

Thus, significant strengthening in the following motivational induction characteristics was observed: Achievement Motive, Affiliation Motive, Help Motive, Development Motive, and Cognition Motive.

The changes were connected with self-attitude and self-esteem in the adolescent girls of both groups. The results of changes in self-attitude among the overweight adolescent girls of Group 1 are presented in Fifure 3.

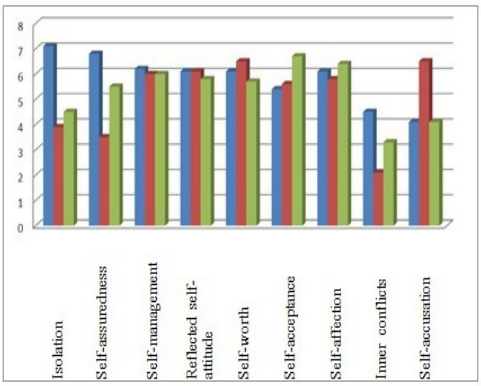

Fig.3. *Changes in self-attitude in the girls of Group 1.*

– Incoming diagnostics; – Intermediate diagnostics; – Final diagnostics

As a result of the application of S.R. Panteleev's research method of self-attitude, the following data was obtained. First of all, overweight adolescents showed a high self-confidence, self-worth, self-acceptance and self-accusation. The data shows that overweight adolescents have a generally positive self-attitude; they demonstrate no aggression and accept themselves. However, they are relatively self-contained, according to the primary diagnostics results.

The intermediate diagnostics results showed a considerable shift in the "Isolation" (P<0.01) and "Self-accusation" (P<0.01) indicators in all adolescents. During the counseling, the patients became more open, but self-criticism expressed in self-accusation also increased. The "Self-acceptance" indicator also decreased, though not so much (P<0.05). It was revealed that in the course of counseling, the overweight adolescent girls became more self-critical and accepted themselves to a lesser degree. With self-attitude development, the adolescents' focus of attention shifted from the outer side of the personality to its inner side, from more or less random traits to the personality as a whole. This shift is connected with realizing their drawbacks, of both inner and outer nature. On the other hand, without changing self-attitude no somatic changes are likely to occur.

The final diagnostics showed primarily stabilization in subjects' self-esteem in the course of counseling. The "Self-accusation" indicator considerably decreased (P<0.01), and the "Self-acceptance" indicator, on the contrary, increased (P<0.01). The data shows that the adolescents have developed a more positive perception of themselves and their bodies.

Changes in self-attitude and and self-esteem of the girls of Group 2 is presented in Fig. 4.

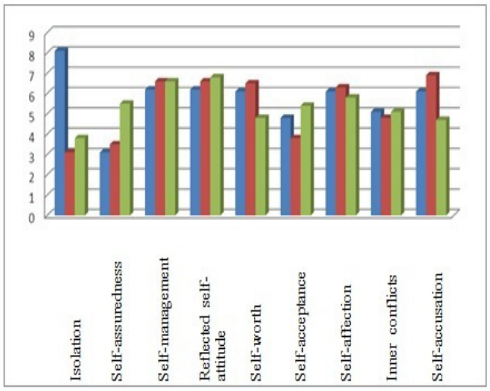

Fig.4. *Changes in self-attitude in the girls of Group 2.*

– Incoming diagnostics; – Intermediate diagnostics; – Final diagnostics

The primary diagnostics results showed practically no difference between the girls of both groups, according to Stolin-Panteleev's test. The only difference observed was the difference in the "Self-accusation" indicator (P<0.05). Thus, the girls of Group 2 showed a higher self-accusation level. The increased values show that the girls blamed themselves for their failure to lose weight and get well. They felt insecure, more isolated, and distrustful.

Intermediate diagnostics using S.R. Panteleev's method of researching self-attitude showed various patterns for adolescents of Groups 1 and 2. In particular, it has turned out that the "Inner conflicts" indicator in the girls of Group 1 remained actually the same (P<0.05). In fact, the girls of Group 2 undergoing treatment repeatedly seemed to have kept a bad

experience, which led to an inner conflict. Similar differences were observed for the "Self-acceptance" indicator (P<0.05). The girls of Group 2 were less prone to self-acceptance than the girls of Group 1. The dynamics is presumably connected with the feeling of successful treatment. The girls undergoing the treatment repeatedly felt less successful because of the bad experience they had in the past.[8,14,16,17]

The final diagnostics data shows that subjects' self-esteem had stabilized. The "Self-accusation" indicator considerably decreased (P<0.01), and "Self-acceptance" increased (P<0.01).

Thus, significant strengthening of the following characteristics of personality self-attitude was observed: self-confidence, self-management, self-attitude, and self-acceptance. On the other hand, isolation, self-accusation and internal conflicts significantly decreased.

The obtained results allow us to correlate them with the studies of other scientists. Various aspects of obese adolescents were considered, such as types of eating behavior, the quality of life in general, complex treatment of adolescents with obesity, and psychological characteristics. There are studies of adolescent motivation, but we haven't found a specific study of the motives of overweight adolescents to receive treatment. The urgency of the problem of changing the overweight adolescents motives to receive treatment, on the one hand, and its underdevelopment, on the other hand, determined the choice of the research topic.

Conclusion

Changes in the motives of overweight adolescents to receive treatment consisted in 1) replacing the directly acting motives with motives arising from the awareness of the goal of the treatment process; 2) in changing the hierarchy of motives, so among the leading motives, the motives of treatment and health acquired significance; 3) in stabilizing the motives of treatment; and 4) in strengthening the success motive.

The overweight adolescents girls admitted to hospital for the first time, by contrast with the girls undergoing their treatment repeatedly, showed more pronounced positive changes in the treatment motives.

Based on the characteristics of motives in the adolescents of the 2 groups, we have assembled a psychological profile of overweight girls, which is characterized by the following:

-Motivation to receive treatment is characterized by decreasing Achievement Motive with simultaneous strengthening of Affiliation Motive, Help Motive, and Self-Development Motive. The adolescents became more focused on pro-social motives. No significant difference in the motivation level in the adolescents of Groups 1 and 2 was observed. Achievement Motive prevailed slightly in Group 1, but this difference was not statistically significant.

-The motivation to receive treatment and interest in the treatment appeared after several consultations; the adolescents became interested in the treatment process itself; they began to actively take part in all psychological exercises, and the treatment motivation appeared. The attending physician noted a positive attitude in the adolescents to treatment.

-In the sphere of body image and eating behavior, we found a significant shift in satisfaction with body image. However, it mainly manifested itself in the girls of Group 1. The girls undergoing treatment repeatedly showed less satisfaction with their changes. In the course of counseling, the adolescents showed significant changes in their assessment of their eating behavior. In particular, such indicators as "Striving for thinness," "Bulimia," "Inefficiency," and "Mistrust in interpersonal relationships" decreased. Specially organized counseling contributed to positive dynamics in the development of eating behavior in overweight adolescents.

-In terms of personality traits, significant shifts were recorded for the "Isolation" and "Self-accusation" indicators in all adolescents. During the counseling, the adolescents became much more open to the psychologist, and at the same time, self-criticism expressed in self-accusation increased. The "Self-acceptance" indicator also decreased. After starting work, overweight adolescents became more self-critical and less self-accepting. This change was associated with their awareness of their internal and external imperfections. On the other hand, no somatic changes are likely to occur without changes in self-attitude.

Thus, a specially developed counseling process comprising diagnostic, informational, correctional and developmental aspects of working with adolescent girls individually and in groups contributed to changing motivation to receive treatment by overweight adolescent girls. Based on the results of the study, we plan to develop a comprehensive system of psychological rehabilitation aimed at developing motivations to receive treatment by overweight adolescent girls.

Competing Interests

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Adolescent Health

INTERNATIONAL JOURNAL OF BIOMEDICINE

Art Therapy for Treating Anxiety in Adolescents with Psychosomatic Diseases

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Abstract

The purpose of our study was to develop and test a program for treating anxiety and hostility in adolescents with psychosomatic diseases (PSDs) by means of art therapy.

Methods and Results: This study included 20 patients with PSDs aged between 15 years and 17 years. The patients' examination included clinical data analysis, using standard records, and testing, using a questionnaire to reveal the severity of psychopathological symptoms (SCL–90–R, adapted by Tarabrina et al. 1997) and the Bekhterev Institute Personality Questionnaire (BIPQ, 1980). Art therapy sessions were conducted from 11:00 a.m. till 1:00 p.m., which coincides with the transition in the daily rhythm of autonomic nervous system activity and is more suitable for the reserve capabilities of the body. The course consisted of 10 daily 1.5-hour procedures. The controlled study duration was 10±2 days. The examination showed that adolescents with PSDs had an above-normal "Anxiety" (ANX) indicator, according to the SCL-90-R method, and a high score for the "Anxious" type (AT) of attitude toward the disease, according to the Bekhterev Institute Personality Questionnaire (BIPQ).

Conclusion: Art therapy for treatment of adolescents with PSD contributed to a normalization of their emotional state, helped them gain new experience in solving problems, and improved the quality of life. (**International Journal of Biomedicine. 2020;10(4):453-456.**)

Key Words: psychosomatic diseases • anxiety • adolescents • art therapy

Introduction

Currently, psychosomatic diseases (PSDs) are quite common among adolescents. Treatment of these diseases is not an independent medical discipline; it is an approach that takes into account a variety of causes that have led to the disease.[1,2] According to the World Health Organization (WHO), 38% to 42% of people in this age group apply to general clinics because of this disorder. A number of authors point to the role of increased anxiety in the development of PSD.[3,4]

In the scientific literature, anxiety is defined in different ways. According to one approach, anxiety is an emotional state. According to another one, it is a personality trait characterized by stability and a relative invariability throughout a person's life. But at the heart of both, according to Spielberger, Khanin, and Kislovskaya, there is the expectation of a threat to a person's own personality. This can lead to constant stress, depletion of bodily resources, and hostility toward others,[5-8] which hinders effective treatment.

It should be noted that according to current literature, one's self-concept is formed during the teen years.[9,10] This means that in these years, knowledge about ourselves, other people and the world around us as a whole (cognitive component) is formed, and on this basis, a certain attitude and behavior toward the above is established (affective and behavioral components). Therefore, it is important to correct anxiety in adolescents with PSD to prevent an unfavorable emotional state from turning into a stable personality trait.

Such diseases require more than pharmaceutical treatment, because medication is not enough to cope with the disease completely; relapses will follow; therefore, the treatment should be accompanied by professional psychological work on the problem.

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Many authors consider that this is possible using art therapy, which allows, in a short period of time, adolescents with PSD to improve their psycho-emotional state, to show and realize their emotions, to get a socially acceptable experience of their expression and control, etc.[11-13]

The purpose of our study was to develop and test a program for treating anxiety and hostility in adolescents with PSD by means of art therapy.

Materials and Methods

This study included 20 patients aged between 15 years and 17 years: 5 of them suffered from vegetative-vascular dystonia and arterial hypertension, 5 had bronchial asthma, 5 - arthritis, and 5 – neurodermatitis. The diagnoses were verified on the basis of findings obtained as a result of clinical, laboratory and functional studies, as well as a psychological examination.

The patients' examination included clinical data analysis, using standard records, and testing, using a questionnaire to reveal the severity of psychopathological symptoms (SCL–90–R, adapted by Tarabrina et al. 1997) and the Bekhterev Institute Personality Questionnaire (BIPQ, 1980). Art therapy sessions were conducted from 11:00 a.m. till 1:00 p.m., which coincides with the transition in the daily rhythm of autonomic nervous system activity and is more suitable for the reserve capabilities of the body. The course consisted of 10 daily 1.5-hour procedures. Controlled study duration was 10±2 days.

The study was approved by the Ethics Committee of the Scientific Center for Family Health and Human Reproduction Problems. Written informed consent was obtained from each research participant (or the participant's parent/guardian).

Statistical analysis was performed using the statistical software package SPSS version 21.0 (IBM Corp., Armonk, N.Y., USA). Continuous variables were presented as mean±standard deviation (SD). The Wilcoxon criterion was used to compare the differences between the paired samples. 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 and Discussion

In the first stage of the study, we diagnosed anxiety in adolescents with PSD and attempted to find its origin. It was discovered that they had an above-normal "Anxiety" (ANX) indicator, according to the SCL-90-R method, being equal to 1.08 and the "Anxious" type of attitude to the disease, according to BIPQ, being equal to 5 points (Fig.1).

Our participants were probably anxious and suspicious about their disease and possible complications. However, this is connected not with real complaints, such as high blood pressure, weakness, headaches, heart pains, etc., but with their focus on interpersonal relationships. This is confirmed by the strong negative correlation between ANX and Somatization (SOM) scales (r=-1, $P<0.01$), as well as the strong positive correlation between ANX and Interpersonal Sensitivity (INT) (r=1, $P<0.01$), according to the SCL-90-R method, AT, according to the BIPQ, and INT, according to the SCL-90-R method (r=1, $P<0.01$) (Fig.2). Based on the characteristics of adolescence described by V. Mukhina,[12] and the presence of a disease that imposes certain restrictions on young people, we believe that their worries may be associated with the desire to be like their peers, who, for example, can walk around in their free time and eat fast food, while the subjects have to stay in bed and keep to a special diet, take medications, and undergo medical procedures. Their desire to be independent and separated is accompanied by knowing that they do not have their own means to purchase medicines, sometimes lack the strength and ability to take care of themselves on their own, and have to follow the doctors' prescriptions.

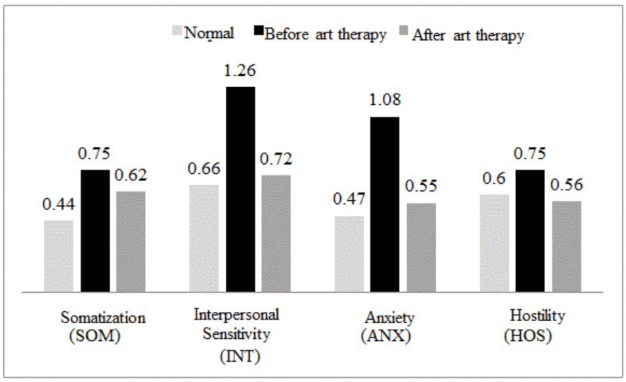

Fig. 1. *Histogram of the severity of individual psychopathological symptoms revealed using the SCL-90-R method before and after art therapy (the results are presented as mean score).*

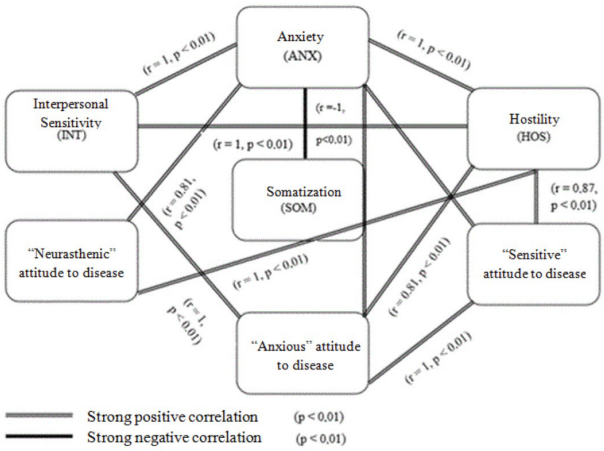

Fig. 2. *Correlation pleiade*

Besides, in our opinion, the need for hospital treatment and the inability to imitate the lifestyle of their peers not only creates tension in the body due to illness, but also anxiety about their own inferiority and the unwillingness of other people to communicate with them. This process is evidenced by the strong correlation between ANX, according to the SCL-90-R method, and the "Sensitive" type (ST) of attitude to the disease, according to the BIPQ (r=1, $P<0.01$) (Fig. 2). It is

most likely due to the importance of intimate and personal communication with other adolescents and the mental polarity of those surrounding our subjects, who can be either kind or very cruel. This means that they can criticize them, or spread rumors about the disease, while subjects of this age, according to V. Mukhina,[12] are very insecure.

Apart from interacting with peers, adolescents with PSD may experience anxiety about their own "inferiority" in relations with their parents. This is evidenced by the second diagnosed type of attitude toward the disease, according to the BIPQ, ST - 4 points (Fig.3). Perhaps the subjects believe that they will bring difficulties to their people; for example, the parents will have to spend time on accompanying them to procedures, and money on medicines for them.

Based on the above, the subjects may show irritability and anger toward others. In our opinion, such a reaction most likely occurs due to weakness, severe physical discomfort, treatment failures and unfavorable examination data, pain and resentment for being sick while others are healthy, fear of becoming dependent on others and not returning to the lifestyle and plans existing before the onset of the disease. This opinion is confirmed by the last type of attitude toward the disease, according to the BIPQ, revealed in the subjects—"Neurasthenic" - 4.5 points—and the strong correlation between the scale "Hostility" (HOS) and INT, according to the SCL-90-R method (r=1, $P<0.01$; Fig. 2), as well as HOS, according to the SCL-90-R method, and ST according to the BIPQ (r=0.87, $P<0.01$) (Fig.3).

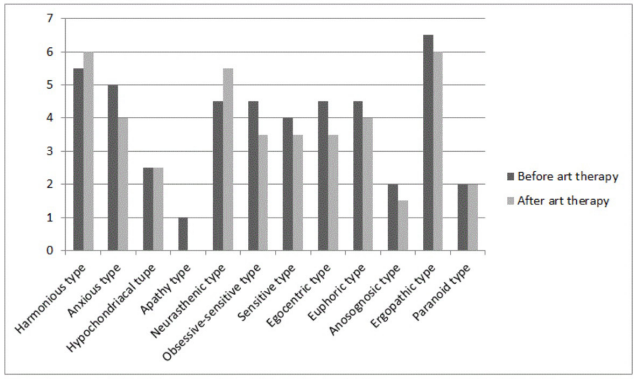

Fig. 3. *Histogram of average values of attitude towards the disease according to BIPQ before and after art therapy*

In the second stage, we conducted 10 group art therapy sessions for 1.5 hours each. The number of meetings and duration of sessions was chosen while taking into account in-patient treatment conditions of the subjects.

In the beginning, the participants introduced themselves and said a few words about themselves. Then the collaboration rules were introduced, and they did an exercise where everyone had the opportunity to individually communicate with all the other members of the group in turn, in order to show what could and couldn't be done when interacting with them. Thus, we tried to bring the subjects closer together and create a trusting contact between them.

Art therapy itself began with working on one's own name (the "Name" technique), since everyone had one, and this created a certain support for the subjects. In addition, it allowed them to discover many personal resources depending on different forms of their names.

Then we worked on the most difficult feelings for each participant, and all participants shared with the group their thoughts, conclusions and emotions arising from such feelings. This made it possible to re-experience and better understand them, gain experience of socially acceptable expression and relieve psycho-emotional stress.

In this connection, at the third stage we analyzed the presence of changes in the results obtained using the Wilcoxon rank sum test after art therapy and found no statistically significant differences (z=0.014, $P<0.05$). We can assume that the psychological factors in the development of PSD in the subjects were acquired a long time ago, have a more complex mechanism, and require more prolonged therapy.

However, quantitative and qualitative analysis shows that the ANX indicator, according to the SCL-90-R method (1.08 before and 0.55 after art therapy), and the score for AT, according to the BIPQ (5 before and 4 after art therapy), decreased and was next to normal. In our opinion, these results are due to the fact that the therapeutic work of the subjects in a group of peers with the same disease helped them share their experiences and learn that others were experiencing similar difficulties, which contributed to an understanding that their difficulties were normal, and enabled them to talk about their ways of coping with anxiety, i.e. relieve emotional stress and gain new experience in solving problems.

Thus, quantitative and qualitative analysis show changes in ANX, according to the SCL-90-R method, and the number of points for AT, in accordance with the BIPQ, after art therapy; however, these findings were not confirmed using the mathematical statistical methods (z=0.014, $P<0.05$). Due to the short duration of the art therapy course (10-12 sessions), the obtained results are probably short-term; therefore, in the future we plan to increase the duration of art therapy and check its effect on anxiety in adolescents with PSD, which will improve their quality of life and adaptive capabilities, and increase their level of social maturity, all of which may reduce the risk of their socio-psychological maladjustment in the future. The study results can be applied in the work of practical psychologists and in psychological counseling practice.

Competing Interests

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

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Association of Serum Uric Acid Levels with Components of Metabolic Syndrome: A Cross-Sectional Analysis in a Saudi Adult Population

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Abstract

Background: This study aimed to investigate the possible relations between serum uric acid (UA) and metabolic syndrome (MetS) and its components in a Saudi adult population sample.

Methods and Results: This cross-sectional study enrolled consecutive adult MetS and non-MetS subjects (90 subjects in each group). Glycemic control indices (fasting blood sugar (FBS), HbA1c, insulin, HOMA-IR index), lipid profile/ratios, and renal function tests were also evaluated.

Findings showed that serum UA was significantly higher in the MetS group (322±98.9) than non-MetS group (286±61.2) µmol/L. The fourth quartile of serum UA showed a higher frequency of MetS (73.3%) and central obesity (82.2%), and higher mean values of triglycerides and FBS as well as lower mean values for HDL-C relative to the first quartiles. Data stratification by sex showed consistent associations of BMI, abdominal obesity, HDL-C, TG/HDL-C, and serum creatinine levels with serum UA in both men and women. Serum UA at 310 µmol/L concentration might be a good predictor for MetS/its components in men. In contrast, at a cut-off level of 275.0 µmol/L, it could significantly predict only obesity and high FBS in women.

Conclusion: Serum UA levels are associated with MetS and may predict MetS and/or its components at specific levels in a sex-dependent pattern in the study population. (**International Journal of Biomedicine. 2020;10(4):457-466.**)

Key Words: metabolic syndrome • uric acid • lipid profile • insulin resistance • Saudi adults

Abbreviations

AUC, area under curve; **BMI**, body mass index; **BP**, blood pressure; **BUN**, blood urea nitrogen; **CRE**, serum creatinine; **FBS**, fasting blood sugar; **HbA1c**, glycated hemoglobin; **HDL-C**, high-density lipoprotein cholesterol; **HOMA-IR**, Homeostasis Model Assessment – IR index; **IDL-C**, intermediate-density lipoprotein cholesterol; **IR**, insulin resistance; **IGT**, impaired glucose tolerance; **LDL**, low-density lipoprotein; **LDL-C**, low-density lipoprotein cholesterol; **MetS**, metabolic syndrome; **TC**, total cholesterol; **TG**, triglycerides; **UA**, uric acid; **VLDL-C**, very low-density lipoprotein cholesterol; **WC**, waist circumference

Introduction

Uric acid (2,6,8-trioxypurine) is the final oxidation product of purine metabolism. The formation of uric acid (UA) is through the enzyme xanthine oxidase, which oxidizes oxypurines (xanthine and hypoxanthine) (https://druginfo.nlm.nih.gov/m.drugportal/rn/69-93-2). For a long time, UA was just considered as a risk for the development of gout and kidney stones.[1] Since the 1900s, however, the accumulated evidence, based mainly on epidemiological studies, has linked serum UA levels to metabolic syndrome (MetS), chronic kidney disease, and cardio-cerebrovascular events.[2-4] A growing body of evidence has suggested that UA may not only be considered as a risk factor of MetS but also an independent predictor of cardio-metabolic diseases and mortality.[1,5,6]

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Moreover, in animal models, decreasing UA levels can prevent or reverse features of MetS.[7, 8]

Although UA can function as an antioxidant, it might be an inexpensive marker of the effects of oxidative stress because its antioxidant activity can be overcome by the pro-oxidant and pro-inflammatory effects on cells.[1, 5] In experiments with cultured vascular cells, UA induces cellular proliferation, inflammation, oxidative stress, and the activation of the local renin-angiotensin system.[1]

In MetS, hyperuricemia has been assigned to hyperinsulinemia and to decline in uric acid excretion associated with kidney dysfunction and is not acknowledged as the main mediator of metabolic syndrome, renal disease, and cardiovascular disorder development. However, more recent investigations have altered this traditional view and shown by providing compelling evidence to support an independent link between hyperuricemia and increased risk of MetS, diabetes, hypertension, kidney disease, and cardiovascular disorders. Despite these emerging findings, controversy regarding the exact role of uric acid in inducing these diseases remains to be uncovered.[6, 9] Interestingly, we have previously identified a high rate of obesity and type 2 DM with their complications.[10, 11] In this sense, this study aimed to investigate the possible relations between serum uric acid (UA) and metabolic syndrome (MetS) and its components in a Saudi adult population sample.

Subjects and Methods

Study population

A total of 180 Saudi adult non-smoker participants aged between 24 and 70 years, presenting at the General Central Hospital, were recruited between June 2017 and December 2017 for this study. All participants were allocated into 2 study groups. The MetS group included 90 individuals with MetS; the non-MetS group included 90 individuals without MetS. MetS was defined according to the revised version of the third report of the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III),[12] i.e. having any 3 of the following 5 diagnostic criteria: (i) Elevated WC (≥92 cm in men and ≥87 cm in women based on the Saudi Abnormal Glucose Metabolism and Diabetes Impact Study (SAUDI-DM);[13] (ii) Elevated TG (≥1.7 mmol/L or on drug treatment for elevated TG; (iii) Reduced HDL-C (≤1.03 mmol/L in men and ≤1.3 mmol/L in women or on drug treatment for reduced HDL-C); (iv) Elevated BP (SBP ≥130 mmHg or DBP ≥85 mmHg or on antihypertensive drug treatment in a patient with a history of hypertension); (v) Elevated fasting glucose (≥5.6 mmol/L) or on drug treatment for elevated glucose.[14,15] Exclusion criteria were participants aged <18 years, pregnancy or breastfeeding, subjects with a history of chronic disease (cardiovascular disease, cancer, stroke, kidney diseases, blood disorders, and gout), treatment with drugs that can affect the results of the study.

Trained nurses through the respective hospital were specified for questionnaire data filling (e.g. age, sex, history of diabetes, hypertension, and dyslipidemia, etc.), anthropometric measurements (height, weight, WC) using Digital Pearson Scale (ADAM Equipment Inc., USA), BP measurement (3 times by using a mercury blood pressure device after the subjects had rested longer than 5 min),[16] and blood sampling following standard protocols to ensure accurate and complete demographic and clinical information for each included participant.

This study was checked against the STROBE (Strengthening the Reporting of OBservational Studies in Epidemiology) checklist and conducted following the "ethical standards of the institutional and national research committee" and with the Helsinki Declaration and its later amendments or comparable ethical standard. It was reviewed and approved by the Medical and Bioethics local committee. Each participant signed informed consent before taking part.

Sample collection and the biochemical analysis

Overnight fast venous blood samples were collected in plain tubes (5 mL) for centrifugation (2500 rpm × 15 minutes) and EDTA tubes (2 mL) for automated glycated hemoglobin (HbA1c) estimation (COBAS, INTEGRA, Roche Diagnostics, USA). The separated sera were divided into aliquots and stored at -80 °C until the time of biochemical analysis. Routine laboratory measurements, including blood urea nitrogen (BUN), serum creatinine (CRE), serum uric acid (UA), fasting blood sugar (FBS), and lipid profile (i.e. TC, HDL-C, LDL-C and TG) were done using commercially available kits on Cobas Integra 400 plus Biochemical analyzer (Roche Diagnostics)". The ratios TC/HDL-C and TG/HDL-C which indicate "the balance between all atherogenic cholesterols (VLDL-C, IDL-C, and LDL-C), and antiatherogenic cholesterol (HDL-C)", were calculated as determinants of cardiovascular risk.[17] Non-HDL-C (i.e. TC – HDL-C) was also calculated as a valuable predictor of premature atherosclerosis and coronary events.[18]

Serum insulin was measured by "Electrochemi-luminescence Immunoassay (Cobas, Roche Diagnostics, USA)" according to the instructions recommended by the manufacturer. Insulin resistance was assessed using HOMA-IR. The calculation formula was as follows: HOMA-IR=(fasting insulin [μIU/mL]×fasting glucose [mmol/L])/22.5.[19, 20]

All the quality control measurements were followed during the laboratory work including running the appropriate calibrators and controls before each run to ensure the performance of the assay.

Statistical analysis

All patient data were coded and anonymized before the analysis. The normally distributed continuous values (i.e. checked by Kolmogorov-Smirnov test) were expressed as mean ±standard deviation (SD) and compared using the Student's t-test (for two groups). One-way analysis of variance (ANOVA) on a rank test (> two groups) followed by Bonferroni multiple comparison tests were also applied. Categorical variables were presented as frequencies (percentages) and compared by Chi-square or Fisher's exact tests. Moreover, Pearson's correlation coefficient was used to test correlations between serum UA and other study variables. Logistic regression analysis was applied to calculate the odds ratios (OR) and 95% confidence intervals (CI) for variables in the study groups, which were adjusted for significant confounding factors as age, parameters of glycemic control, lipid profile, and kidney function test. Receiver operating characteristics (ROC) analysis was used to calculate the area

under the curve (AUC) for serum UA and to find the best cutoff values associated with maximum sensitivity and specificity to identify MetS and its components. Calculation of the study power using "G power-3 software version 3.0.10 (http://www.gpower.hhu.de/)", showed that with the specified study design, and allowable error rate; alpha error = 0.05 with sample size 90 for each group can give 89% power with nearly an effect size = 0.44. The optimal cutoff value for each clinical-laboratory measurement to predict MetS was calculated. Results with $P<0.05$ were considered statistically significant. Data analysis was done by the Statistical Package for the Social Sciences software (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY).

Results

Baseline characteristics of study participants

The baseline clinical and biochemical characteristics of the study subjects were summarized in Tables 1 and 2. Also, the associations of these parameters with MetS components were presented. As shown in Table 1, individuals older than 45 years had higher odds (OR=3.28; 95% CI: 1.76-6.13) of having MetS compared with those less than 45 years old. The odds ratios of central obesity, hypertension, diabetes mellitus, hyperglycemia, dyslipidemia, hypercholesterolemia, hypertriglyceridemia, elevated LDL-C, and increased IR were 5.61, 27.9, 5.20, 4.33, 11.2, 2.21, 14.8, 4.37, and 2.91, respectively, (all $P<0.05$) for risk to develop MetS relative to ones who do not have any of these disorders. As expected, subjects in the MetS group had significantly higher values of glycemic parameters (FBG, HbA1c, insulin, and HOMA-IR index), lipid profile and ratios (TC, TG, LDL-C, TC/HDL-C, TG/HDL-C, and Non-HDL-C), BUN, and UA than ndividuals in the non-MetS group (Table 2).

Prevalence of MetS components among the study participants

Of the 180 study subjects, central obesity was observed in 112(62%) of participants in whom women had 3.8 times susceptibility for getting this MetS component than men (95% CI: 1.96-7.54; $P<0.001$). In contrast, low HDL-C levels were more prominent in men (64%) than women (32%). Other MetS components (i.e. hypertension, hypertriglyceridemia, and IGT) show consistency among men and women (Table 3).

Table 1.
Clinical and biochemical characteristics of the study participants

Variables		Non-MetS	MetS	*P*	OR (95% CI)
Number		(n=90)	(n=90)		
Age	Mean ± SD	37.3 ± 16.3	43.1 ± 12.0	0.004 [b]	
	≤45 years	66 (73.3)	41 (45.6)	<0.001 [a]	Reference
	>45 years	24 (26.7)	49 (54.4)		3.28 (1.76-6.13)
Sex	Male	23 (25.6)	30 (33.3)	0.252 [a]	0.68 (0.36-1.30)
	Female	67 (74.4)	60 (66.7)		
Weight, kg	Mean ± SD	79.3 ± 16.3	90.8 ± 19.1	<0.001 [b]	
Height, cm	Mean ± SD	160.3 ± 8.4	163.3 ± 9.17	0.025 [b]	
BMI, kg/m^2	Mean ± SD	30.9 ± 6.4	33.9 ± 6.6	0.001 [b]	
Abdominal obesity	Negative	51 (56.7)	17 (18.9)	<0.001 [a]	Reference
	Positive	39 (43.3)	73 (81.1)		5.61 (2.86-11.0)
Hypertension	Negative	78 (86.7)	17 (18.9)	<0.001 [a]	Reference
	Positive	12 (13.3)	73 (81.1)		27.9 (12.4-62.4)
Diabetes mellitus	Negative	78 (86.7)	50 (55.6)	<0.001 [a]	Reference
	Positive	12 (13.3)	40 (44.4)		5.20 (2.49-10.85)
High FBS	Negative	78 (86.7)	54 (60.0)	<0.001 [a]	Reference
	Positive	12 (13.3)	36 (40.0)		4.33 (2.06-9.08)
High insulin	Negative	81 (90.0)	74 (82.2)	0.195 [a]	Reference
	Positive	9 (10.0)	16 (17.8)		1.94 (0.81-4.67)
Dyslipidemia	Negative	25 (27.8)	3 (3.3)	<0.001 [a]	Reference
	Positive	65 (72.2)	87 (96.7)		11.15 (3.22-38.5)
High TC	Negative	62 (68.9)	45 (50.0)	0.015 [a]	Reference
	Positive	28 (31.1)	45 (50.0)		2.21 (1.20-4.06)
High TG	Negative	85 (94.4)	48 (53.3)	<0.001 [a]	Reference
	Positive	5 (5.6)	42 (46.7)		14.8 (5.51-40.1)
High LDL-c	Negative	42 (46.7)	15 (16.7)	<0.001 [a]	Reference
	Positive	48 (53.3)	75 (73.3)		4.37 (2.19-8.74)
Low HDL-c	Negative	52 (57.8)	53 (58.9)	0.880 [a]	Reference
	Positive	38 (42.2)	37 (41.1)		0.95 (0.52-1.72)
HOMA-IR	IS	79 (87.8)	64 (71.2)	0.009 [a]	Reference
	IR	11 (12.2)	26 (28.8)		2.91 (1.34-6.35)

[a] *Fisher's Exact or Chi-square tests were used for qualitative variables, and* [b] *Student's t-test was used for quantitative data. According to HOMA-IR all participants were divided into insulin-sensitive (IS) and insulin resistance (IR, >1.9) groups (Vogeser et al., 2007).*

Table 2.
Biochemical characteristics of the study participants

Variables	Non-MetS (n=90)	MetS (n=90)	*P*
FBS, mmol/L	5.6 ± 1.9	6.8 ± 3.1	0.002
HbA1c, %	5.36 ± 1.1	6.7 ± 1.65	<0.001
Insulin, mIU/mL	4.49 ± 2.7	5.82 ± 3.8	0.008
HOMA-IR	1.15 ± 0.9	1.6 ± 1.0	0.001
TC, mmol/L	4.7 ± 1.09	5.2 ± 0.86	0.002
TG, mmol/L	1.14 ± 0.4	1.68 ± 0.9	<0.001
LDL-C, mmol/L	2.9 ± 1.0	3.3 ± 0.8	0.003
HDL-C, mmol/L	1.22 ± 0.3	1.19 ± 0.3	0.583
TC/HDL-C	4.11 ± 1.4	4.69 ± 1.4	0.009
TG/HDL-C	1.0 ± 0.52	1.5 ± 1.07	<0.001
Non-HDL-C	3.57 ± 1.1	4.05 ± 0.9	0.002
BUN, mmol/L	5.13 ± 1.9	6.5 ± 2.2	<0.001
CRE, mmol/L	64.1 ± 14.8	67.4 ± 16.0	0.148
UA, mmol/L	286 ± 61.2	322 ± 98.9	0.004

Table 3.
Prevalence of components of metabolic syndrome among the study participants

Component	Total (n=180)	Men (n=53)	Women (n=127)	*P*	[a]OR (95%CI)
Central obesity	112	21 (39.6)	91 (71.1)	<0.001	3.85 (1.96-7.54)
Hypertension	85	29 (54.7)	56 (44.1)	0.251	0.65 (0.34-1.24)
High TG	47	17 (32.1)	30 (23.6)	0.266	0.65 (0.32-1.32)
Low HDL-C	75	34 (64.2)	41 (32.3)	<0.001	0.26 (0.13-0.52)
IGT	52	16 (30.2)	36 (28.3)	0.857	0.91 (0.45-1.84)

[a]OR (95% CI): adjusted odds ratio for age (95% confidence interval).

Prevalence of MetS and its components among serum UA quartiles

Figure 1 shows the prevalence of MetS and its components among the four quartiles [Q1:< 248.3, Q2: 248.3- <290, Q3: 290- <361.7, Q4: ≥ 361.7) of serum UA.

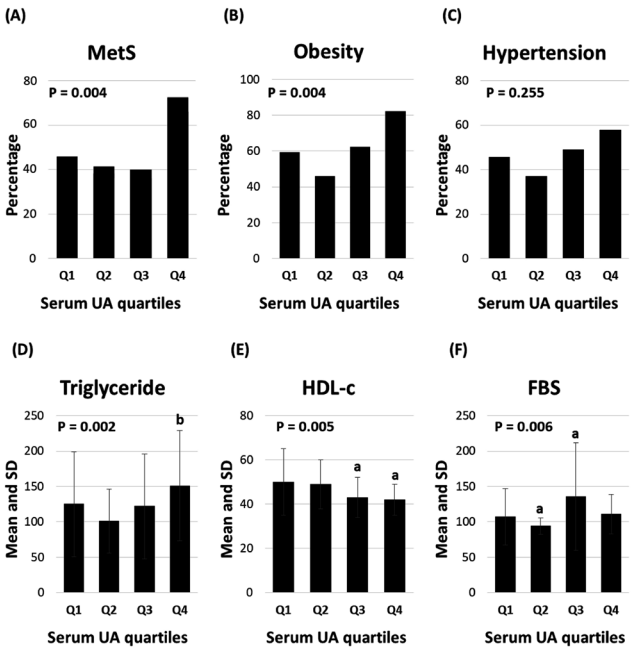

Fig. 1. Overall analysis of the prevalence of MetS and its components by quartiles (Q1-Q4) of serum UA. *Data are presented as percentages (A-C) or mean and SD (D-F).*

Overall, individuals in the Q4 of serum UA showed a significantly higher frequency of MetS (73.3%) and central obesity (82.2%) relative to the ones in the Q1-Q3 (UA-Q1 to UA-Q3; 45.5%, 41.3%, 40.0% of MetS frequency, and 59.1%, 45.7%, and 62.6% of central obesity frequency, respectively).

Furthermore, higher mean values of TG and FBS and lower mean values for HDL-C were observed in UA-Q4 relative to the first ones (Table 4). Data stratification by sex showed a central obesity prevalence increase in UA-Q4 for both males and females. At the same time, subjects with higher mean TG levels and lower mean HDL-C levels were more frequent in UA-Q4 of men relative to that in women (Table 4).

Association between serum UA and the clinical-laboratory parameters

Correlations between serum UA and the clinical-laboratory parameters are summarized in Table 5. Overall analysis showed that serum UA was correlated with age (r=0.174, *P*=0.019), weight (r=0.318, *P*<0.001), height (r=0.236, *P*=0.001), BMI (r=0.175, *P*=0.018) and abdominal obesity (r=0.216, *P*=0.004). In the current study, we found an inverse correlation between serum UA and HDL-C levels (r=-0.259, *P*<0.001) and significant positive correlations between serum UA and other laboratory parameters, including the glycemic variables (FBS, HbA1c, insulin, and HOMA-IR), the lipid profile-related variables and ratios (TC/HDL-C, TG/HDL-C, and non-HDL-C), and kidney function test-related variables (BUN and CRE) as shown in Table 5. Interestingly, data stratification by sex showed consistent associations of BMI, abdominal obesity, HDL-C, TG/HDL-C, and serum creatinine levels with serum UA in both men and women.

Evaluation of serum UA concentration and other clinical-laboratory parameters in diagnosing MetS and its components

An overall analysis has revealed that serum UA at a cut-off point of 295.05 µmol/L could predict MetS, central obesity, high levels of TG, and low HDL-C in the total study participants (Figure 2).

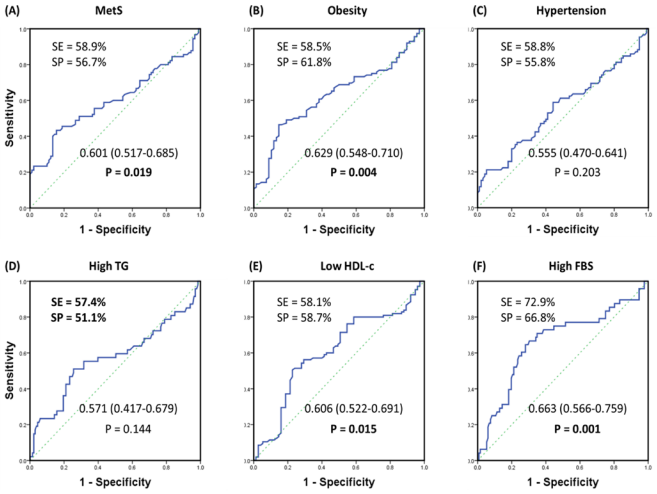

Fig. 2. An overall analysis of the ROC curve for serum UA concentration predicting MetS and its components. *(A) Metabolic syndrome, (B) Central obesity, (C) Hypertension, (D) Hypertriglyceridemia, (E) Low HDL-C, (F) High FBS.*

Table 4.

Prevalence of MetS and its components by quartiles (Q1-Q4) of serum UA stratified by sex

Component	UA-Q1	UA-Q2	UA-Q3	UA-Q4	*P*
Overall					
MetS	20 (45.5)	19 (41.3)	18 (40.0)	33 (73.3)	0.004
Central obesity	26 (59.1)	21 (45.7)	28 (62.2)	37 (82.2)	0.004
Hypertension	20 (45.5)	17 (37.0)	22 (48.9)	26 (57.8)	0.255
TG	1.4 ± 0.8	1.14 ± 0.5	1.4 ± 0.8	1.7 ± 0.9 [b]	0.002
HDL-C	1.3 ± 0.4	1.3 ± 0.3	1.1 ± 0.2 [a]	1.1 ± 0.2 [a]	0.005
FBS	6.0 ± 2.2	5.2 ± 0.7 [a]	7.6 ± 4.2 [a]	6.2 ± 1.6	0.006
Men (n=53)					
MetS	4 (57.1)	4 (40.0)	8 (44.4)	14 (77.8)	0.139
Central obesity	2 (28.6)	0 (0.0)	8 (44.4)	11 (61.1)	0.014
Hypertension	2 (28.6)	5 (50.0)	11 (61.1)	11 (61.1)	0.455
TG	1.3 ± 0.5	0.9 ± 0.4	1.5 ± 90.8	2.0 ± 1.1	0.008
HDL-C	1.3 ± 0.2	1.4 ± 0.1	1.1 ± 0.2	1.1 ± 0.2	0.002
FBS	5.4 ± 1.0	5.4 ± 0.6	9.6 ± 5.8	5.9 ± 1.3	0.359
Women (n= 127)					
MetS	16 (43.2)	15 (41.7)	10 (37.0)	19 (70.4)	0.055
Central obesity	24 (64.9)	21 (58.3)	20 (74.1)	26 (96.3)	0.007
Hypertension	18 (48.6)	12 (33.3)	11 (40.7)	15 (55.6)	0.313
TG	1.4 ± 0.9	1.2 ± 0.5	1.3 ± 0.7	1.5 ± 0.6	0.107
HDL-C	1.3 ± 0.4	1.2 ± 0.3	1.1 ± 0.3	1.1 ± 0.2	0.084
FBS	6.1 ± 2.4	5.2 ± 0.7	6.2 ± 1.9	6.4 ± 1.8	0.006

Data are presented as frequency (percentage) or mean and SD. Pearson Chi-square and Kruskal-Wallis tests were applied, followed by Bonferroni multiple comparison test. a: statistically significant relative to UA-Q1, b: statistically significant relative to UA-Q2.

Table 5.

Correlation analysis between serum UA level and the clinical-laboratory parameters

Clinical data	Total subjects	Men	Women
Age, years	0.174 (0.019)	-0.056 (0.693)	0.230 (0.009)
Weight, kg	0.318 (<0.001)	0.555 (<0.001)	0.274 (0.002)
Height, cm	0.236 (0.001)	0.312 (0.023)	0.083 (0.354)
BMI, kg/m^2	0.175 (0.018)	0.495 (<0.001)	0.178 (0.046)
Abdominal obesity	0.216 (0.004)	0.446 (0.001)	0.249 (0.005)
Hypertension	0.095 (0.204)	0.235 (0.090)	0.006 (0.946)
Diabetes	0.122 (0.104)	0.071 (0.612)	0.114 (0.200)
Dyslipidemia	0.063 (0.402)	0.006 (0.963)	0.139 (0.118)
Laboratory tests			
FBS, mmol/L	0.221 (0.003)	0.119 (0.395)	0.260 (0.003)
HbA1c, %	0.243 (0.001)	0.025 (0.858)	0.343 (<0.001)
Insulin, mIU/mL	0.169 (0.023)	0.361 (0.008)	0.088 (0.325)
HOMA-IR	0.246 (0.001)	0.392 (0.004)	0.147 (0.100)
TC, mmol/L	0.094 (0.207)	0.439 (0.001)	-0.004 (0.964)
TG, mmol/L	0.243 (0.001)	0.432 (0.001)	0.148 (0.097)
LDL-C, mmol/L	0.122 (0.103)	0.380 (0.005)	0.059 (0.510)
HDL-C, mmol/L	-0.259(<0.001)	-0.459 (0.001)	-0.200 (0.024)
TC/HDL-C	0.243 (0.001)	0.481 (<0.001)	0.165 (0.063)
TG/HDL-C	0.299 (<0.001)	0.464 (<0.001)	0.213 (0.016)
Non-HDL-C	0.167 (0.025)	0.467 (<0.001)	0.071 (0.427)
BUN, mmol/L	0.211 (0.004)	0.049 (0.726)	0.210 (0.018)
CRE, mmol/L	0.502 (<0.001)	0.646 (<0.001)	0.427 (<0.001)

Data are presented as the correlation coefficient (P-values).
Abdominal obesity: WC ≥92 cm in men and ≥87 cm in women.

On stratified analysis by sex, serum UA at approximately 310 μmol/L concentration was found to be a good predictor for MetS and all its components in men. While at a cut-off level of 275.0 μmol/L, serum UA could significantly predict obesity and high FBS among all MetS components in women [AUC (95%CI): 0.659 (0.566-0.753), and 0.691 (0.522-0.831), respectively] (Table 6).

On investigating the optimal cutoff values of the clinical-laboratory parameters in the prediction of MetS, the BMI was the best anthropometric measurement to predict MetS [AUC (95% CI): 0.689 (0.609-0.769)] with sensitivity (80%) and specificity (60%) which moderately minimizes the false-positive/-negative cases. The positive likelihood ratio (PLR) was equivalent to 2.0, suggesting that those subjects with BMI ≥30.4 kg/m^2 may present approximately twice the chance of a positive diagnosis being true. In contrast, the negative likelihood ratio (NLR) corresponded to 0.33, which is close to three times the chance of a negative diagnosis confirming the absence of MetS. The PLR and NLR of UA were 1.36 and 0.72, respectively, at 295 μmol/L serum level (Table 7).

Discussion

Given the growing prevalence of obesity, prediabetes, and diabetes, [21, 22] the study of factors that interplay with MetS and/or its components becomes an essential area of public health concern.

Table 6.

Evaluation of serum UA concentration in diagnosing MetS and its components stratified by sex

Variable	AUC	95% CI	S	*P*	Cut-off (mmol/L)	SE	SP	LR+	LR-
OVERALL									
MetS	0.601	0.517-0.685	0.04	0.019	295.0	58.9%	56.7%	1.36	0.752
Obesity	0.629	0.548-0.710	0.04	0.004	295.0	58.5%	61.8%	1.53	0.67
Hypertension	0.555	0.470-0.641	0.04	0.203	295.0	58.8%	55.8%	1.33	0.73
High TG	0.571	0.417-0.679	0.05	0.144	295.0	57.4%	51.1%	1.17	0.83
Low HDL-C	0.606	0.522-0.691	0.04	0.015	295.0	58.1%	58.7%	1.40	0.71
High FBS	0.663	0.566-0.759	0.04	0.001	295.0	72.9%	66.8%	2.19	0.40
Men									
MetS	0.675	0.531-0.820	0.07	0.030	310.5	66.7%	61.9%	1.75	0.54
Obesity	0.763	0.626-0.901	0.07	0.001	310.5	81.0%	62.5%	2.16	0.30
Hypertension	0.636	0.486-0.787	0.08	0.090	310.5	62.1%	54.2%	1.36	0.70
High TG	0.798	0.652-0.944	0.07	0.001	310.5	82.4%	58.3%	1.98	0.30
Low HDL-C	0.783	0.661-0.905	0.06	0.001	310.5	84.2%	61.8%	2.20	0.26
High FBS	0.536	0.377-0.696	0.08	0.660	310.5	65.0%	51.5%	1.34	0.68
Women									
MetS	0.561	0.457-0.665	0.05	0.237	275.0	58.3%	44.8%	1.06	0.93
Obesity	0.659	0.566-0.753	0.05	0.005	275.0	63.7%	61.1%	1.64	0.59
Hypertension	0.504	0.398-0.609	0.05	0.946	275.0	53.6%	40.8%	0.91	1.14
High TG	0.451	0.323-0.579	0.07	0.420	275.0	50.0%	41.2%	0.85	1.21
Low HDL-C	0.608	0.505-0.711	0.05	0.050	275.0	62.8%	56.1%	1.43	0.66
High FBS	0.691	0.522-0.831	0.07	0.002	275.0	75.0%	49.5%	1.49	0.51

S: standard error, SE: sensitivity, SP: specificity, LR+: positive likelihood ratio [=SE/(1-SP)], LR-: negative likelihood ratio [=(1-SE)/SP].

Table 7.

The optimal cut-offs of clinical and laboratory variables in the prediction of MetS

Variable	AUC	95% CI	S	*P*	Cut-off (mmol/L)	SE	SP	LR+	LR-
Age, years	0.634	0.551-0.716	0.04	0.002	40.5	62.2%	64.4%	1.75	0.59
Weight, Kg	0.693	0.615-0.771	0.04	<0.001	82.5	66.7%	61.1%	1.71	0.55
Height, cm	0.590	0.507-0.673	0.04	0.037	160.5	57.8%	59.0%	1.41	0.72
BMI, kg/m^2	0.689	0.609-0.769	0.04	<0.001	30.4	80.0%	60.0%	2.00	0.33
UA, mmol/L	0.601	0.517-0.685	0.04	0.019	295	58.9%	56.7%	1.36	0.72
FBS, mmol/L	0.625	0.542-0.707	0.04	0.004	5.5	52.2%	73.3%	1.96	0.65
HbA1c, %	0.673	0.594-0.752	0.04	<0.001	5.0	65.6%	57.8%	1.55	0.60
Insulin, mlU/mL	0.598	0.515-0.680	0.04	0.024	4.29	54.4%	53.4%	1.17	0.85
HOMA-IR	0.664	0.585-0.743	0.04	<0.001	1.23	56.7%	63.3%	1.54	0.68
TC, mmol/L	0.633	0.551-0.715	0.04	0.002	4.97	62.2%	60.0%	1.56	0.63
TG, mg/dL	0.666	0.587-0.745	0.04	<0.001	1.3	54.4%	66.7%	1.63	0.68
LDL-C, mmol/L	0.648	0.565-0.730	0.04	0.001	3.14	56.7%	65.6%	1.65	0.66
HDL-C, mmol/L	0.514	0.429-0.598	0.04	0.753	1.16	50.0%	51.1%	1.02	0.98
TC/HDL-C	0.614	0.532-0.696	0.04	0.008	4.2	61.1%	60.0%	1.53	0.65
TG/HDL-C	0.660	0.580-0.741	0.04	<0.001	1.0	57.8%	60.0%	1.45	0.70
Non-HDL-C, mmol/L	0.635	0.554-0.717	0.04	0.002	3.8	60.0%	64.4%	1.69	0.62

S: standard error, SE: sensitivity, SP: specificity, LR+: positive likelihood ratio [=SE/(1-SP)], LR-: negative likelihood ratio [=(1-SE)/SP].

The present study evaluated the associations of serum UA levels with MetS and its components in a sample of adult Saudi residence in the Northern area of Saudi Arabia. This region, as part of the "Middle East and North African (MENA)" region, is known for its high prevalence of MetS as recently supported by Al-Rubean et al.' s study, which revealed "a prevalence of 39.9% at specifically 45.0% in men and 35.4% in women" according to the same criteria the authors applied in the present study (i.e. NCEP ATP III criteria).[16] The authors preferred to apply the latter criteria rather than that of the International Diabetes Federation (IDF) [23] which mandates the presence of central obesity as one of the MetS components, contributing to apparently less disease prevalence and missing of several risky individuals who have other MetS components.

Currently, participants older than 45 years showed three times the odds of having MetS compared with those less than 45 years old. This could be attributed in part to the association of the age with the increased central obesity, hormonal changes, and IR,[24,25] as well as the analogous increase in the prevalence of specific MetS components (e.g. diabetes and hypertension) with age in the current population.[26,27]

In the present study, the serum UA level was higher in the MetS group compared to non-MetS one. This finding was consistent with the previous studies in other areas of the world.[28-32] Although serum UA has been reported to have a potent antioxidant capacity (i.e. conferring nearly 50%-60% of the total plasma antioxidant activity) in the circulation,[33] it can promote oxidative and inflammatory stress in the intracellular context[34-36] by several mechanisms detailed previously[37-39] which lead to endothelial cell dysfunction and contribute to MetS biogenesis.[34-36]

The overall analysis revealed that participants in the fourth quartile of serum UA showed a significantly higher frequency of MetS and central obesity relative to the ones in the first quartiles. This finding could support the mutual relations that could be present between serum UA and MetS/ components, which have been debated recently.[40] Chronic hyperuricemia found to be implicated in the pathogenesis of metabolic perturbation that could lead to MetS. Its control may prevent or reverse the course of MetS and/or its components.[41,42] Previously, Zhang et al.'s longitudinal cohort study, and a study by Chen et al. revealed that individuals with a higher concentration of serum UA, have 1.6 times the risk of developing MetS.[43,44] Furthermore, Nejatinamini et al. showed that higher serum UA levels, "even within the normal ranges", were associated with increased odds to have MetS (nearly double risk per one unit increment in serum UA), and could be considered as one of the determinants of the MetS.[31] Our findings are also consistent with that of Choi and Ford 's study on a nationally-representative sample of US adults.[45] They found that MetS prevalence and its individual components increase with increasing grades of hyperurecemia, and they recommended more intensive clinical investigation for a potential coexistence of the MetS in cases presented with high serum UA levels.[45] The close relation of hyperuricemia with MetS might be supported by the fact that UA biosynthesis is linked to glycolysis that is regulated by insulin. A decrease in the glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase (GA3PDH) activity in case of insulin resistance leads to shifting of glucose utilization from glycolysis into other pathways like the pentose phosphate pathway which is the main source of ribose-5-phosphate, the building unit of phosphoribosyl pyrophosphate (PRPP), which participates in the synthesis of nucleic acids including the purines and, consequently, increases its degradation products and the emergence of hyperuricemia.[46] In this respect, the latter mechanism could also explain the current finding of the high frequency of central obesity in high UA level quartiles as it is known that the visceral fat to be specifically associated with more insulin resistance than other types of adiposity due to the combined effect of increase free fatty acids released and adipokine secretion deregulation.[47]

Although overall correlation analysis showed that serum UA was correlated with age, BMI, and abdominal obesity, stratification analysis by sex revealed a consistent correlation of both BMI and central obesity with serum UA in both sexes. Still, a significant correlation with age was only evident in females. Interestingly, this finding is in line with recent findings of Sun et al., who revealed correlations between UA, hyperuricemia, and coronary artery diseases only in females, but not in men.[48] Additionally, several previous studies in different areas of the world supported this association like the National Health and Nutrition Examination Survey (NANHES) on the US general population,[49] the Italian Pro. V. A. study[50] and the community-based study in China.[51] Although the exact cause of this association is still undefined, the postmenopausal decline of the protective estrogens might explain in part this relation and the consistent correlation of serum UA with the glycemic variables (FBS and HbA1c %) in the present enrolled women.[52]

The present study identified an inverse correlation between serum UA and HDL-C levels and significant positive correlations between serum UA and other laboratory parameters, including the lipid profile-related variables and ratios (TC/HDL-C, TG/ HDL-C, non-HDL-C, and TG/HDL-C) in overall analysis with consistent associations with HDL-C and TG/HDL in both men and women after stratification by sex. This finding expands the previous studies that correlate serum UA with dyslipidemia and an increase in the risk of developing high LDL-C and hypertriglyceridemia.[53,54] The TG/HDL-C ratio is known to be a practical approach for identifying individuals who have insulin resistance, as concluded previously.[55,56] Given the role of HDL-C in the reverse transport of cholesterol from the peripheral tissues to the liver, the inhibitory effect on LDL oxidation, attenuation of platelet aggregation, and stimulation of prostacyclin secretion, HDL-C has a protective role against atherogenicity.[57] As previously indicated that it is hard to estimate the "LDL particle size" increment, which is associated with the hypertriglyceridemia risk,[58] TG/ HDL ratio is considered an accurate and putative marker for atherogenicity,[55] and prediction of the cardio-metabolic risk.[59, 60]

Remarkably, on testing the predictive capacity of serum UA to predict MetS and/or its components, the current results were largely in line with the available literature concerning

adult subjects.[61, 62] These studies including ours, suggest that hyperuricemic individuals could develop MetS at specified cut-off values among different sexes. It is noteworthy that these cut-off values (310 μmol/L and 275.0 μmol/L in the enrolled men and women, respectively) are less than those proposed for the gout treatment[63], which can decrease the chance of missing individuals with high risk to cardiovascular diseases (including MetS individuals) as supported by Cicero et al.[62] Although overall serum UA discriminating value was not the best one among other predictors for MetS, its cut-off point was associated with moderate sensitivity and specificity values, which minimize the false-positive and the false-negative included cases.

Collectively, it is evident that there is a significant association of serum UA levels with MetS and its components, including those associated with an increase in the risk of cardiovascular diseases. At specific cut-off levels, serum UA might predict MetS and/or its components in a sex-specific pattern in the study population that deserves great concern for careful monitor and control. It is worth noting that some limitations should be considered. The study design is a cross-sectional with a modest sample size that enrolled patients with relatively health awareness who were routinely attending the Medicine outpatient clinics at the general hospital, which could add some source of selection bias as it does not represent the general population. Not all the confounding factors could be adjusted (e.g. the physical activity, nutritional status, etc.) as the related data were not complete. Also, the direct causal relation of UA with MetS could not infer from the current study. Longitudinal follow-up studies with larger sample size and with different ethnicities are recommended.

Conclusion

The study findings suggest that levels of serum UA in the Saudi population might be associated with the risk of MetS and its components. In this sense, modifying the lifestyle and early management of hyperuricemia could be a useful strategy for lowering the MetS burden in this region. It is highly recommended to explore the causal relationship between hyperuricemia and MetS in future large-cohort studies.

Competing Interests

The authors declare that they have no competing interests.

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Cigarette Smoking and Components of the Metabolic Syndrome in the Indigenous Population of the Arctic Territory of Yakutia

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Abstract

The aim of this research was to study the influence of smoking on components of the m*etabolic syndrome* and their relationship in the indigenous population of the Arctic territory of Yakutia.

***Methods and Results*:** The material for the study was collected under expeditionary conditions in the Arctic territory of Yakutia in places of compact residence of indigenous peoples. Using the continuous method, we examined 348 subjects (225 women and 123 men). The average age of the respondents was 48.16±0.52 years (49.71±0.63 years for women and 44.98±0.91 years for men). The research program included the following sections: a questionnaire for objective assessment of state; anthropometric examination; assessment of blood pressure (BP); determination of insulin and fasting glucose. In the survey, only those who smoked at least 1 cigarette per day during the last 12 months were considered smokers. The obtained results indicate that the indigenous population of the northern territory of Yakutia has a high frequency of risk factors for the development of cardiovascular pathology, such as overweight, obesity, and smoking. Overweight is equally common in both men and women; obesity is almost 2.5 times more common in women. An increase in body weight and systolic BP is not associated with smoking; however, the simultaneous combination of all these factors can affect the risk of cardiovascular incidents. A negative relationship between body mass indexand smoking was found. An increased index of insulin resistance is associated with the simultaneous spread of obesity among nonsmokers. The same association of glucose level with BP was obtained in both smokers and nonsmokers.

Conclusion: Metabolic disorders in the indigenous population of the northern territory of Yakutia are caused by a change in the traditional way of life and nutrition, with a decrease in physical activity and a lack of adherence to a healthy lifestyle. (**International Journal of Biomedicine. 2020;10(4):467-471.**)

Key Words: smoking • overweight • obesity • insulin • glucose • systolic blood pressure • indigenous population • Yakutia

Introduction

The prevalence of overweight and obesity is a global epidemic affecting both developed and developing countries.[1-3] Numerous researchers have demonstrated that there is a relationship between obesity and insulin resistance. Obesity is generally thought to lead to hyperinsulinemia based on pathophysiological and metabolic mechanisms. Hyperinsulinemia, in turn, leads to the development of the metabolic syndrome and type 2 diabetes mellitus. The connection between insulin resistance and the risk of developing cardiovascular pathology has been established in many studies.[4-10]

It is generally recognized that tobacco smoking is one of the negative factors affecting the human body.[11,12] According to the WHO, more than a billion people smoke, with a steady increase every year.[13] Russia is one of the top countries with a high prevalence of tobacco smoking.[14] Scientists have received conflicting results on the effect of smoking on body mass index (BMI). For example, scientists have found that nicotine, acting on the levels of various neurotransmitters, such as catecholamines, dopamine, and serotonin, suppresses appetite and, therefore, reduces food intake, which results in a lower BMI.[15,16] A meta-analysis of prospective cohort studies in China, Singapore, and the United States showed

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that smokers with type 2 diabetes mellitus had an increased relative risk of cardiovascular complications and death.[17] The relationship between BMI and insulin levels under the direct or indirect influence of tobacco smoking is currently widely discussed in the scientific community.[18,19] Previously, results were published on the high prevalence of overweight and obesity among the indigenous peoples of the northern territory of Yakutia.[20] Given the change in the traditional lifestyle and nutrition, the study of the relationship between smoking, obesity, and insulin levels in this population remains relevant and poorly understood.

The aim of this research was to study the influence of smoking on components of the m*etabolic syndrome* and their relationship in the indigenous population of the Arctic territory of Yakutia.

Materials and Methods

The material for the study was collected under expeditionary conditions in the Arctic territory of Yakutia in places of compact residence of indigenous peoples (Nizhnekolymsky District, Verkhnekolymsky District, Tomponsky District). Using the continuous method, we examined 348 subjects (225 women and 123 men). Patient sample consisted of an adult population aged from 20 years to 70 years. The response rate was 70%. Average age of the respondents was 48.16±0.52 years (49.71±0.63 years for women and 44.98±0.91 years for men).

Inclusion criteria: representatives of indigenous peoples of Yakutia (the Yakuts, the Evens, the Chukchi, the Yukagir). Exclusion criteria: representatives of non-indigenous nationality and the Yakuts. The sample was formed according to the administrative lists of employees of the settlements. The response was 76%.

The research program included the following sections: a questionnaire for objective assessment of state; informed consent of the respondent to conduct research and donate blood; anthropometric examination; and blood sampling from the cubital vein in the morning on an empty stomach, with 12-hour abstinence from food. After centrifugation, blood serum was stored in a freezer (-70°C) until analysis. In the survey, only those who smoked at least 1 cigarette per day during the last 12 months were considered smokers.

Overweight was considered to be a BMI≥25and<30kg/m², obesity was determined at a BMI of ≥30 kg/m².

Laboratory methods included the determination of insulin and fasting glucose. The generally accepted HOMA-IR index was used to calculate insulin resistance:[21,22] (Fasting blood insulin in mIU/mL × Fasting blood glucose in mmol/L)/22.5. An index value exceeding 2.7 is considered insulin resistance.

Blood pressure (BP) was measured twice with an OMRON M2 Basic automatic tonometer, with subjects in a sitting position. Average BP was calculated with a margin of permissible measurement error of ±3mmHg, according to the instructions for the correct measurement of BP outlined in the European clinical guidelines for the diagnosis and treatment of hypertension. The diagnosis of hypertension was based on 2017 ACC/AHA Guideline for or the Prevention, Detection, Evaluation, and Management of High Blood Pressure in Adults. [23]

The study was approved by the Ethics Committee of the Yakut Science Center of Complex Medical Problems. Written informed consent was obtained from each patient.

Statistical analysis was performed using IBM SPSS Statistics for Windows, Version 19.0 (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. Baseline characteristics were summarized as frequencies and percentages for categorical variables and as mean±SEM for continuous variables. 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. The frequencies of categorical variables were compared using the Chi-square test. Spearman's rank correlation coefficient 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 and Discussion

In the general population, almost half of the respondents (45.7%) were found to smoke; men had the largest percentage of smokers - 59.3%, women - 38.2%.

The average BMI was significantly higher in women than in men (28.73±0.42 kg/m² and 25.51±0.36 kg/m², respectively, $P<0.001$). In the general population, 35.9% of the respondents were overweight, and 31.9% were obese; 36% of women were overweight, 39.6% were obese. In men, 35.8% and 17.9%, respectively. Thus, an equally high incidence of overweight was found in both men and women. Obesity is most common among the female population. The high incidence of overweight, BMI and obesity is due to changes in lifestyle and low physical activity, especially among women.

We conducted a study on the relationship between smoking and BMI. In nonsmokers, BMI was statistically significantly higher than in smokers (28.93±0.44 kg/m² and 26.01±0.39 kg/m², respectively, $P<0.001$). In nonsmoking men, as well as in women, the mean BMI was significantly higher than in nonsmokers (men: 27.20±0.58 kg/m² and 24.36±0.41 kg/m², $P<0.001$; women: 29.55±0.55 kg/m² and 27.42±0.60 kg/m², $P=0.013$). A negative relationship between BMI and smoking has been confirmed in many studies;[15,16] nevertheless, it has been shown that smoking is an independent risk factor for the development of cardiovascular diseases, including stroke and coronary heart disease.[12,24-26]

Table 1 presents a comparative analysis of overweight and obesity in the general population, depending on gender and on the status of smoking. The frequency of overweight in the general population of smokers was higher than in nonsmokers due to the higher rate of obesity among female smokers; however, the differences are statistically insignificant. With regard to obesity, all respondents had a significantly higher incidence of obesity in nonsmokers than in smokers. In our study, smoking did not affect the development of constitutional obesity, which is confirmed by the studies

of several researchers on the effect of nicotine on weight loss through an increase in various neurotransmitters.[15,16]

Table 1.

Frequency of overweight and obesity by BMI depending on status of smoking (%)

		Overweight	P-value	Obesity	P-value
Total	smokers	37.7	0.587	20.8	0.001
	nonsmokers	34.4		41.3	
Men	smokers	32.9	0.412	9.6	0.042
	nonsmokers	40.0		30.0	
Women	smokers	41.9	0.122	30.2	0.039
	nonsmokers	32.4		45.3	

Comparing the systolic BP (SBP) level among the respondents, depending on their smoking status, the following results were obtained. In smokers, the average SBP was significantly lower than in nonsmokers (134.84±1.85 mmHg and 147.83±1.98 mmHg, respectively, $P<0.001$), while the average SBP in nonsmokers was higher than normal. There was also a negative correlation between the number of smoked cigarettes and the level of SBP (r=-0.226, $P<0.001$); the statistically significant correlation was obtained in women (r=-0.220, $P=0.001$) than in men (r=-0.147, $P=0.104$). Accordingly, 64.2% of nonsmoking respondents had a significantly increased BP, compared with smokers, whose BP increased in 35.8% of cases ($P<0.001$). Thus, we obtained results in which smoking is not associated with an increase in BP, possibly due to its indirect effect on BMI reduction. Results obtained by Li et al. [27] also revealed that the adjusted BP was lower in current smokers versus nonsmokers and former smokers. Nevertheless, according to previous research, the combination of these two risk factors affects the mortality rate from cardiovascular complications.[12,17,24-26,28,29]

Due to conflicting research data on the effect of smoking on the level of insulin secretion, we carried out a comparative analysis of the average values of insulin and the HOMA-IR index in respondents, depending on their relationship with smoking. The average insulin concentration did not exceed the reference values in smokers; it was 7.19±1.23 IU/ml, which was significantly lower than in nonsmokers (12.74±2.35 IU/ml) ($P=0.035$). By gender, only men had significant differences in mean insulin concentration (4.91±0.75 IU/ml in smokers and 8.42±1.76 IU/ml in non-smokers, $P=0.034$). There were no significant differences in women. We also determined the average values of the HOMA-IR index, which were 1.48±0.24 in smokers and 2.77±0.46 in nonsmoking respondents ($P=0.014$). In nonsmokers, these indicators were higher than the reference values. Thus, our study shows that smoking suppresses insulin secretion, which may affect the development of type 2 diabetes. Similar results were obtained in the studies of certain foreign researchers.[18,19] The increased HOMA-IR index in nonsmoking respondents, which characterizes insulin resistance, is most likely associated with the prevalence of obesity among them.

When comparing the relationship between the number of smoked cigarettes and BMI, we obtained a statistically significant negative correlation (r=-0.318, $P<0.001$). This correlation was more clearly observed in men (r=-0.423, $P<0.001$) than in women (r=-0.134, $P=0.088$). With insulin, a weak inverse relationship was obtained with the number of cigarettes smoked (r=-0.140, $P=0.029$). Separately, in men and women, there was no clear relationship (men: r=-0.163, $P=0.153$; women: r=-0.140, $P=0.029$). We did not find any particular relationship between the number of cigarettes smoked and the glucose level (r=-0.045, $P=0.489$). Separately, in men and women, there was no correlation either. The obtained result does not reflect the influence of one of the risk factors on the development of atherosclerosis and type 2 diabetes mellitus, since we have not determined the duration of smoking. Thus, the number of cigarettes smoked was not associated with BMI and insulin levels.

We analyzed the association of insulin, glucose, SBP and BMI parameters under the direct influence of smoking, and we studied the relationship between insulin and the level of SBP in the general population. There was a weak positive correlation between insulin levels and SBP (r=0.239, $P=0.003$), which is confirmed by studies abroad.[6,8,31] Analysis of the effect of smoking on the relationship between insulin and SBP showed that nonsmokers had a significant but weak correlation (r=0.197, $P=0.034$), in contrast to smokers (r=0.116, $P=0.198$).

A study of the relationship between insulin levels and BMI showed that there was a positive correlation between these indicators (r=0.283, $P<0.001$). Depending on the smoker's adherence to smoking, the level of insulin was significantly correlated with BMI, equal to the correlation for nonsmokers (r=0.283 and r=0.223, respectively, $P<0.05$ in both cases). As for the relationship between glucose level and BMI, a weak correlation was obtained in nonsmokers (r=0.287, $P=0.002$), but not in smokers (r=0.031, $P=0.731$). Glucose was also associated with SBP in both smokers (r=0.183, $P=0.021$) and nonsmokers (r=0.420, $P<0.001$). Thus, an association between the insulin level and BMI was obtained, including in smokers, which is confirmed by a number of studies abroad.[4, 8,18]

In summary, the indigenous population of the northern territory of Yakutia has a high frequency of risk factors for the development of cardiovascular pathology, such as overweight, obesity, and smoking. Overweight is equally common in both men and women; obesity is almost 2.5 times more common in women. An increase in body weight and SBP is not associated with smoking; however, the simultaneous combination of all these factors can affect the risk of cardiovascular incidents. We have confirmed the data of foreign scientists on the suppression of the level of insulin secretion under the influence of nicotine. An increased index of insulin resistance is associated with the simultaneous spread of obesity among nonsmokers. The same association of glucose level with BP was obtained in both smokers and nonsmokers. Metabolic disorders in the indigenous population of the northern territory of Yakutia are caused by a change in the traditional way of life and nutrition, with a decrease in physical activity and a lack of adherence to a healthy lifestyle. By addressing modifiable

risk factors, including overweight, obesity, and smoking, it is possible to prevent morbidity and premature mortality from cardiovascular disease.

Competing Interests

The authors declare that they have no competing interests.

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CASE REPORT

INTERNATIONAL JOURNAL OF BIOMEDICINE

Stepwise Treatment of Uterine Arteriovenous Malformation Complicated by a Giant Aneurysm: A Case Report

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Abstract

Uterine arteriovenous malformation (AVM) is a rare condition that threatens severe uterine bleeding. We present a patient with this pathology, who was hospitalized twice within 5 months in serious condition, because she refused a hysterectomy. Ultrasonography of the uterus detected a vascular formation, the nature of which could be identified only on computed tomography (CT) angiography. Afferent vessels were embolized twice, and after the condition was stabilized, we performed a hysterectomy. For a long time, there was no recurrence of bleeding. The foregoing allows us to recommend the described tactics for the diagnosis and treatment of uterine AVM in women in similar clinical situations. (**International Journal of Biomedicine. 2020;10(4):472-474.**)

Key Words: arteriovenous malformation • embolization • aneurysm • vaginal bleeding

Introduction

Uterine arteriovenous malformation (AVM) is a very rare pathology, but potentially a life-threatening condition.[1] Usually, uterine AVM manifests by periodic profuse uterine bleedings, pelvic pain, and anemia.[2] Uterine AVMs are classified into congenital and acquired anomalies. Acquired anomalies are more common and usually follows a history of previous uterine trauma, such as curettage procedures, caesarean section, traumatic delivery, pregnancy termination, manual placenta removal, forceps delivery, vacuum extraction, or pelvic surgery.[4-6] The acquired uterine AVMs often demonstrate complex and numerous arteriovenous communications, which may be due to the rich vascular network within the uterus. The initial imaging modality of choice for AVM is sonography with color and spectral Doppler analysis.[7] The gold standard for diagnosis of uterine AVMs is digital subtraction angiography.[8]

Case Presentation

A 37-year-old woman in serious condition was admitted to the hospital with complaints of fatigue and copious spotting. Anamnesis showed that she had spotting for three years, with increased frequency and intensity during the last year, after separate diagnostic curettage. Hemoglobin level at admission was 58 g/L, the platelet count was $101\times10^3/\mu L$. The patient underwent a blood transfusion, after which the hemoglobin level increased to 83 g/L. Ultrasound imaging showed enlargement of the uterus to 140 mm × 107 mm and vascular mass in the myometrium closer to the left side wall with a diameter of 95 mm. In the digital duplex mapping mode, signs of turbulent blood flow with possible thrombotic masses along the periphery were detected (Fig.1 A,B).

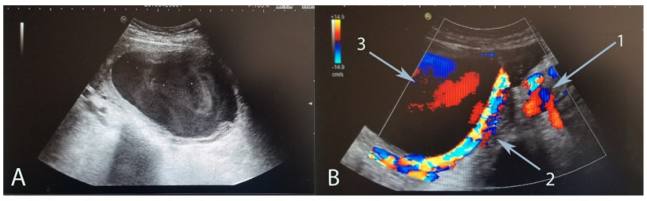

Fig. 1. *(A) Ultrasound image shows vascular formation with a diameter of 95 mm located in the myometrium. (B) Color Doppler: 1. Dilation of the afferent and efferent vessels; 2. Parietal rapid blood flow from a large afferent; 3. Turbulent blood flow in the aneurysm cavity.*

The preliminary diagnosis was posttraumatic pseudoaneurysm after the traumatic diagnostic curettage. To clarify the diagnosis, CT angiography was performed. It revealed a vascular formation at the level of the iliac vessels on the left — a partially thrombosed aneurysm of a small vessel, arteriovenous malformation.

Despite the young age, the patient was offered a hysterectomy. Due to a serious health condition, in order to stop bleeding and prepare for surgery, the initial embolization of uterine arteries was performed. Selective angiography revealed dilated, convoluted vessels (the left uterine and the right uterine), with a contrast discharge from the distal segments into the cavity of the false aneurysm (Fig.2 A, B, C). During full contrasting, numerous collaterals between the the right uterine and left uterine were visualized. Further, these arteries with spirals were embolized. On the control angiogram, blood flow in these vessels was reduced, and contrast discharge into the aneurysm was not observed. The postoperative period was normal, uterine bleeding was stopped. The patient was discharged in satisfactory condition and was transferred to another hospital to have a hysterectomy; however, a hysterectomy was not performed.

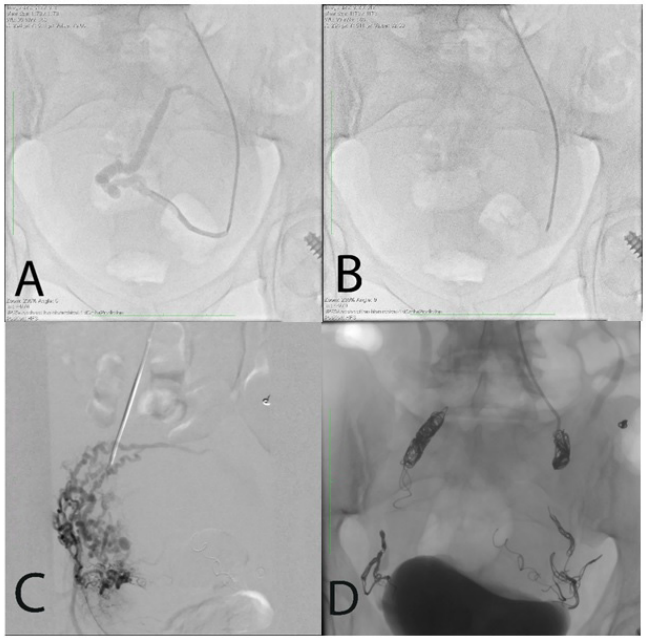

Fig.2. *(A) Selective angiography: A large afferent branch of vascular malformation; (B) Selective angiography: the contrast agent enters the aneurysm; (C) The expanded convoluted right uterine artery with numerous collaterals in the area of the left uterine artery and the discharge of contrast from the distal segments into the cavity of the false aneurysm; (D) Control angiography: The embolization of the left uterine artery, right uterine artery, and left ovarian artery.*

After 5 months, the patient was again hospitalized in a severe condition with a recurrence of uterine bleeding, weakness, dizziness, and tinnitus. The heart rate was 120 bpm, blood pressure - 100/60 mmHg. Blood hemoglobin was 36 g/L; platelet count was reduced to 76×10^3/μl. After hemotransfusion, the hemoglobin level increased to 89 g/L. Duplex scanning revealed an aneurysm measuring 90 mm × 87 mm × 80 mm with parietal thrombotic masses in the left wall of the uterus with a visible blood flow. In the region of the left internal iliac artery, there were multiple arterial branches with the main type of blood flow and venous collaterals. A CT scan of the small pelvis with contrast demonstrated the formation of a spindle-shaped form 42 mm × 28 mm in size with lacunar filling with a contrast agent in the arterial phase on the left between the internal and external iliac arteries — a partially thrombosed aneurysm of a small vessel, arteriovenous malformation.

The patient underwent an intervention to stop bleeding. On selective angiograms of the right and left iliac arteries, hypertrophic afferent branches of malformation from obstructive, cystic, and internal genital arteries were contrasted. All afferent vessels with spirals for vascular embolization were embolized, with the additional injection of a crushed hemostatic sponge. Additionally, we performed bilateral occlusion of the internal iliac arteries with a tight arrangement of vessels with spirals for embolization (Fig.2 D). On control angiography, blood flow through the vessels was reduced; malformation was not contrasted.

The postoperative period was normal. The patient was transferred to a specialized department to have a hysterectomy. After 10 days, a hysterectomy was successfully performed. After another week, the patient was discharged in satisfactory condition. The vaginal discharge did not recur.

Discussion

Traditionally, uterine AVM has been diagnosed after hysterectomy using the histopathological examination. In this case, due to the morphological features of the pathology, only a computed tomography of the small pelvis with contrast allowed us to establish the correct diagnosis. It should be noted that selective digital subtraction angiography, which in such cases should be considered as the gold diagnostic standard, gives a complete picture of the formation angioarchitectonics.[8,9] Due to the patient's initial serious condition, a hysterectomy was associated with an extremely high risk of death. In our opinion, the preliminary use of X-ray endovascular techniques, including bilateral internal iiac artery embolization, made it possible to stop bleeding rapidly, minimize intraoperative blood loss, and avoid fatal complications. Thus, the foregoing allows us to recommend the described tactics for the diagnosis and treatment of uterine AVM in women in similar clinical situations.

Competing Interests

The authors declare that they have no competing interests.

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