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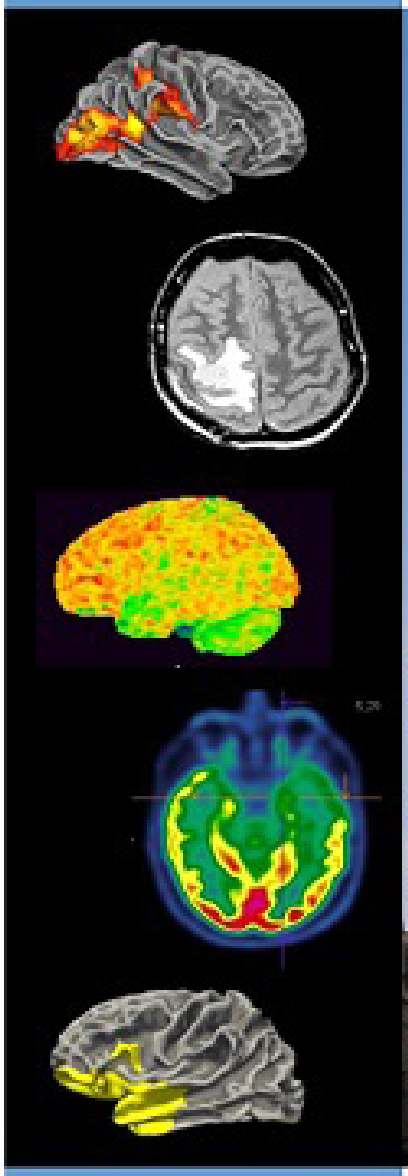

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REVIEW ARTICLE

INTERNATIONAL JOURNAL OF BIOMEDICINE

Biomedical Research and Healthcare: Opportunities, Expectations, and Limitations

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Abstract

The last fifty years have been the "golden era" of biomedical research and innovation. Major discoveries in genetics, genomics and various fields of "Omics", together with the technology revolution, has created unlimited opportunities for the development, and improvements in the way the healthcare is delivered. Not a single day goes by, without an announcement of a new sensor, new app, or a new and novel technology, that can be integrated with the wealth of knowledge in biomedical research and applications. To the extent, one of the largest insurance provider, John Hancock announced, that they no longer offer policies, that do not include digital tracking. They will sell only "interactive" policies that collect health data through wearable devices, such as smart watch. The breakthroughs in biomedicine, and advances in technologies, have been miraculous. This is especially true in the USA, which is the envy of other nations, when it comes to innovations in research and technology. The fact that all of these innovations are "news makers" creates great expectations from the care receivers. Having said that, patients, clinicians, and healthcare providers feel at times a letdown, or question the slow pace of advance, escalating cost, sometimes dubious clinical values and inappropriate exploitations. Policy makers and economists are debating, about the cost-effectiveness and the return on the investment in biomedical research, as it relates to improvements in health care. Researchers worldwide are debating about the availability of "Precision Medicine" and "Personalized Medicine." Despite the developments in biomedical research and emerging technologies, which have raised our expectations and created infinite opportunities, there seems to be some limitations in their applications. In this mini review, we will briefly discuss some of the developments in biomedical research and innovation. We will also express our views on the opportunities available and explain limitations. (**International Journal of Biomedicine. 2018;8(4):273-279.**)

Key Words: biomedical research • healthcare • technology innovations • genomics

Introduction

Measuring the contribution of biomedical education and research is more or less a guess than a true estimate. It has been estimated that 23-48% of the decline in mortality over the 1930-1978 is attributed to biomedical research efforts. It translates to a net return of 83 billion, illustrating the wisdom of investment in biomedical education and research.[1] For example, biomedical research in the U.S. is a over 100-billion-dollar enterprise, - 65% supported by the industry and 35% by the National Institutes of Health. The dilemma regarding the return on investment is compounded by the unpredictable nature of basic sciences and its applications.[2] In the "State of the Union" address, President Barack Obama on the 20th of January 2015 made the following announcement; "Tonight I am launching a new precision medicine initiative, to bring us closer to curing diseases like cancer and diabetes- and to give all of us, access to the personalized information we need to keep ourselves and our families healthier." Dr. Francis Collins, the director National Institutes of Health, USA announced an initiative called, "All of US", a billion-dollar program, which has two main components: a near-term focus on cancers and a longer-term aim to generate knowledge applicable to the whole range of health and disease.[3] The NIH researchers envisage to recruit a "cohort" of 1 million Americans, who will consent to give biologic specimens (cell populations,

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proteins, metabolites, RNA, DNA-including whole-genome sequencing and behavioral data, all linked to the electronic health records.

If you do a search on the top ten biomedical innovations, you hardly get any discussions on biomedical education, research or applications. Cleveland Clinic at their 15th annual medical innovation summit in October 2018 list the following: Artificial pancreas, pacemaker, gene therapy, reduction in LDL, new generation vaccines, breast cancer therapies etc. Since the discovery of the double helix nature of the DNA (1951-53), biology has evolved into a global industry promising miraculous biomedical applications and opening the door to precision and personalized medicine. The Human Genome Project launched a new area of "BIG Science," It was 13-year-long publicly funded project initiated in 1990, with two key principles; 1) welcomed all collaborators from any nation, 2) required all human genome sequence information should be freely, publicly, available. With the initial success in this project in 2002, first successful genome-wide association study (GWAS) was published for studying myocardial infarction.[4] With the advances in the knowledge of molecular cloning, gene transfer in the 1980s, molecular medicine emerged as a novel and revolutionary therapy.[5] After decades of ups and downs, according to an article in Science, "return of gene therapy" had a breakthrough in 2009. In brief, the gene therapy is quite simple; a functional copy of the defective gene is introduced to replace the missing function or a defective gene.[6] Two decades after the initiation of gene therapy trials with more than 1700 approved clinical studies, first therapy product (Glybera) approved by European Medical Association, is available for use in the European Union for the treatment of ADA deficiency.[7]

Cellular therapies and regenerative medicine, with great potential to improve the health of patients, represent a game changer in modern healthcare delivery, by focusing on the underlying causes of the disease by repairing, replacing, or regenerating damaged cells, organs and tissues. First allogenic hematopoietic stem cell transplant (HSCT) was done more than fifty years ago in a patient suffering from acute leukemia. Fifty years after this clinical breakthrough, HSCT remains the only stem cell therapy widely used in clinical practice.[8] Researchers, clinicians, and biotechnologists worldwide, are investigating ways and means to mitigate, the challenges and risks of stem cell therapy. Embryonic stem cells are promising, but there are challenges when it comes to controlling the cell growth. Several laboratories are testing mesenchymal stem cells (MSCs), as they can be isolated from any adult tissue, in addition to fetal tissue and cord blood. Due to the lack of a single marker to define MSCs derived from different sources, the regulatory bodies have adopted a criteria, regarding marker expression and differentiation potential.[9] In the area of regenerative medicine, the University of Minnesota researchers developed "Ghost Hearts" and claimed that a real beating bio-artificial heart will be ready in a few years.[10]

Doris Taylor and associates at the University of Minnesota, pumped detergents through rat hearts, and obtained a biological scaffold (ghost heart), for an artificial heart that comprised of collagen and other extra cellular matrix. They incubated it in a bioreactor and reseeded it, with heart cells from a newborn mouse. Results of this pioneering study were published in the Nature Medicine.[10] Although tissue engineered hearts are not yet available, the techniques developed have been of great use in cardiac repair.[11] Developments in the biological sciences, cannot really compete with the rapid progress that is taking place, in emerging technologies and biomedical innovations. Just a few years ago, it was unthinkable, that any body part can be printed with synthetic components. It has now become a reality. Researchers at the University of Minnesota have 3D printed a bionic eye. The device is an array of semiconductor photodetectors, made of polymers, printed on glass hemisphere.[12] Scientists in Switzerland have 3D printed a silicone heart, that works and pumps like a real human heart.[13] Researchers in Netherland have developed 3D-printed tooth that has antibacterial properties. Canadian researchers have used 3D printing, to develop skin that is tissue specific to patients for wound healing applications.

We mentioned that developments in basic science applications are unpredictable. White in his article on the history of Diabetes, mentions that management of diabetes during the past several thousand years, since time of Pharoh's (3500 ago) to the present, has changed considerably.[14] Despite these observed changes in the management of this metabolic disease, it continues to increase even at the time of this writing in unprecedented rate. Metabolic diseases such as, hypertension, excess weight, obesity, and diabetes (type 2) have reached epidemic proportions worldwide.[15-20] According to these experts, obesity has doubled (over a billion) and diabetes has increased four-fold worldwide in the last three decades. A multi-country review on the global prevalence of diabetes concluded, "Most people with diabetes live in low- and middle-income countries and these will experience, the greatest increase in diabetes for the next 22 years. Countries like India and China, with very large populations, have had an increase of two-fold to 17-fold in diabetes incidence in the last three decades. Framingham Heart Studies, initiated some 70 years ago in the USA, developed basic information on the modifiable risk factors for developing cardiovascular diseases (CVDs). In a Science editorial, Brown and associate speculated that, "Exploitation of recent breakthroughs-proof of the cholesterol hypothesis, discovery of effective drugs, and better definition of genetic susceptibility factors- may end coronary disease as major public health problem.[21] Two decades after this claim was made, cardiovascular disease remains the number one killer worldwide. In this mini review, we will discuss some biomedical innovations, research accomplishments, expectations, and limitations as well as express our viewpoints on these findings.

Discussion

According to the experts, genomics will most likely make its greatest contribution to health by revealing mechanisms of common, complex disease, such as hypertension, diabetes and asthma.[22-25] From the time the Human Genome Project was initiated, there is great expectation and excitement, about its possible contribution to improvements in healthcare. Having

said that, there seems to exist considerable confusion among health care professionals, educators, and public about the exact role of genetic information in medical practice. Dr Francis Collins in an article in N. Engl. J. Med. writes, "If genetics has been misunderstood, genomics is even more mysterious."[22] Genetics is the study of single genes and their effects. Genomics is the study not of single genes, but of the functions and interactions of all the genes in the genome. For instance, human gut microbiota contains tens of trillions of microorganisms, including at least 1000 species of known bacteria with more than 3 trillion genes, which influence human physiology, metabolism, nutrition, immune function, and disruption of normal metabolism. In the large genomic study, we described earlier as a part of the "All of Us' initiative, diabetes is one of the topics of interest. Metabolic alterations such as oxidative stress, chronic inflammation, hypertension, endothelial dysfunction, subclinical atherosclerosis, excess weight, and obesity contribute significantly to the pathogenesis of diabetes and its clinical complications. In a situation like this, we are looking at the individual's gene and its interactions as well as his/her microbiota genes and their combined contribution to the altered metabolic processes.

Several researchers have reported, that metabolic signature of plasma free branched chain and aromatic amino acids, strongly predict future diabetes development.[26-30] Based on this type of investigations, diabetes predictive amino acid score has been developed (DM-AA score). It has been shown, that a score of fasting plasma level of isoleucine, tyrosine, and phenylalanine, predict diabetes development, predicts CVD events during long-term-follow-up.[30] Fuzisaka and associates from Joslin Diabetes Center, Harvard Medical School, performed LC-MS based metabolomic analysis, of cecal contents and plasma metabolites. Of the over 1000 unidentified metabolites, eighteen correlated positively with host insulin resistance.[31] The researchers concluded that, "The changes at the level of gut and blood are dramatically influenced by diet, exposure to antibiotics, genetic background, and site of bacterial colonization. These and other such studies are challenging and hard to interpret, as we are dealing with thousands of gene interaction products and metabolites in the gut and their role in altered amino acid or fatty acid metabolism. Of course, it would be useful to fully understand the complex role of diet, gene expression of the host, the gut microbiota, and the modulating effect of various metabolites, in the initiation and progression of metabolic risks and metabolic diseases.[32]

Type 2 diabetes (T2D) has reached epidemic proportions, worldwide and as such there is lots of interest in genetic studies related to this metabolic disease. The Genome Wide Association Studies (GWAS) has confirmed epidemiological observations of genetic links, between lipid dysregulation and glycemia (FADS1, GCKR, HNF1A), circadian rhythmicity, metabolic derangements (MTNR1B, CRY2), low birth weight and subsequent T2D risk (ADC5). Type-2 diabetes GWAS have been successful in identifying specific loci, that contribute to the causation of the complex disease roughly in only 10% of the heritability suggesting, that much remains to be discovered.[33] Although there is a great hope and expectation that such studies, will provide opportunity for therapeutic interventions, and pharmacogenetic clinical trials, common genetic variants identified so far, are not yet useful in clinical prediction or therapy. In order to find the "missing heritability" researchers are pursuing fine-mapping around the associated regions, leveraging the 1000 genomes project, using next generation sequencing, analyzing the Metabochip, improved informatics for gene x gene and gene x environment interactions.[33]

Recent advances in regenerative medicine has generated great enthusiasm and expectations for various clinical applications and easy cure. Just like the new drug development, cell cultures require Good Manufacture Procedures (cGMP), but cell cultures are more complex and less controlled than small molecule research, common in drug discovery studies. In addition, many challenges exist in todays' highly regulated healthcare environment. There is no harmonization between different regulatory authorities. Stem cell research has a real potential, to have significant impact on human health. There is however great controversy, about the use of human embryos for this kind of work. Scientists have been circumventing this concern, by using a method that can turn adult stem cells into pluripotent stem cells, which can change into any cell type. Despite these advancements, there is still a lot more to be done before the researchers can create successful treatments through stem cell therapy. Stem cell therapies are not new. Clinicians have been performing bone marrow stem cell transplants for at least half a century. The very first successful bone marrow transplant was done in 1956 at Cooperstown New York, by Dr Donnall Thompson in identical twins. The first non-twin (allogenic) transplant was done at the University of Minnesota in 1968.

In early 80s our research group at the University of Minnesota, demonstrated that in drug- induced diabetes animal model, vascular prostaglandin synthesis is altered, to create an imbalance between the thromboxane produced by the platelets, and prostacyclin generated by the vessel wall.[34] The changes observed both in platelet and vascular tissue, were corrected by islet cell transplantation.[34] ViaCyte a company based in San Diego, California, has obtained FDA approval for their product PEC-Direct and has treated its first patient. Via Cyte's PEC-Direct device allows a patient's blood vessels to integrate and contact the transplanted beta cells. VC-01 or PEC-Encap, is an implantable device containing embryonic stem cells that develop into pancreatic progenitor cells. VC-02 or PEC-Direct also transplants progenitors but the device allows patient's blood vessels to integrate with these transplanted cells (direct vascularization). Has the regenerative medicine come of age? Currently there are number of funded clinical trials, studying everything from stroke, to spinal cord injury and HIV/AIDS. It is heartening to note, that FDA has approved Kymirah and Yescarta for chimeric antigen receptor therapy (CAR-T). A type of treatment in which patient's T cells are changed in the laboratory, so they will attack cancer cells when transfused back into the patient. There is lot more to do. Most of these studies are Phase 1, Phase 2 trials. There are just a relatively few Phase 3 clinical trials.

Advances in tissue engineering and regenerative

medicine technologies, created immense opportunities for the development of tissues, organs, and sophisticated grafts for therapeutic applications. Modern era of tissue engineering is relatively young and began only a couple of decades ago. In brief, tissue engineering involved the *ex vivo* engineering of replacement of tissues for subsequent *in vivo* implantation. Skin substitute represented the earliest attempts of engineered tissues. In the early 90s, the stem cell biology began a full-scale emergence, and dedicated Stem Cell Institute and Translational Science Institutes were developed to support these applied biomedical technologies. More than 4,000 people are on the waiting list for a heart transplant in the USA alone, at any given point of time. Doris Taylor, Bakken Professor and the director of the center of Cardiovascular Repair, University of Minnesota, outlined her results on "bioartificial hearts", prior to the publication of her research in 2008 at the "Understanding Aging, Biomedical and Bioengineering Approaches" conferences at UCLA. She seems to have claimed that recellularized human hearts may be weeks away. Popular science went wild with the announcement of new and emerging field of rejuvenation biotechnology. Just at the same time, I was participating in a Stem Cell conference in which, a young investigator was heralding, that in the near future body parts will be available on medical shelves, for replacement of the defective parts.

In brief, the process of developing a beating heart is a simple process. Infuse a strong detergent through a donor heart (rat, mouse or pig), obtain a "ghost heart" with the intact exoskeleton of the donor heart. The scaffolds obtained from donor hearts retain the macro- and micro architecture, vasculature, and biochemical cues for cellular adherence, proliferation and differentiation. Once you have the "ghost heart" from an animal or human source, infuse the donor heart skeleton with millions of blood or bone-marrow stem cells, from a person who needs a heart transplant, place it in a bioreactor- a container with artificial lungs and tubes that pump oxygen, blood or nutrient cocktails into it, wait as the ghost heart matures, and starts beating like human heart (Fig 1).

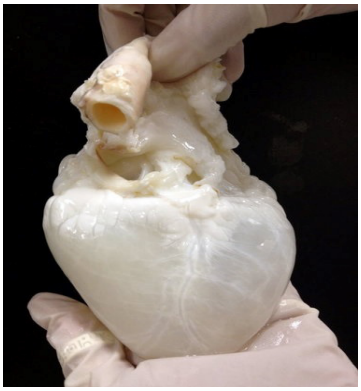

Fig. 1. *Bioartificial Heart (Dr. Taylor).*

According to the researchers at the Division of Cardiovascular Sciences, National Heart, Lung and Blood Institute of the National Institutes of Health (NIH), USA, despite widespread interest in the use of regenerative medicine to improve cardiac function, both in acute myocardial infarction and in chronic heart failure, the clinical benefit has been modest and variable between clinical trials, with some showing no benefits.[35] Just like the efforts to develop a bioartificial heart is facing challenges, 3D printing is also is in its infancy. Using imaging data and a thermoreversible support bath to bioprint an embryonic heart, researchers have developed a novel complex internal and external anatomical structure to mimic a human heart (Fig 2). However, these model hearts lacked the appropriate vasculature. Furthermore, all blood-contacting biomaterials and surfaces used in the development of these bioartificial organs, must be designed to be thrombo-and calcification-resistant in order to be successful post implant.

Fig. 2. *3D-Printed Bioartificial Heart (public: CNN).*

Long before the stem cell research and translational science platforms developed, the researchers at the university of Minnesota, were interested in developing bioartificial pancreas and bioartificial liver. As a young faculty of the Biomedical engineering, I was collaborating with the Center for Interfacial Engineering, a platform, which encouraged multidisciplinary research to encourage integration of emerging technologies. We also had established collaboration with the Medical Device Industries. One such company that we were collaborating at that time, was the Excorp Medical Corp Minneapolis, Minnesota. (http://www.excorp.com/html/product.html).

The product that they have been working on for quite some time is the Bioartificial Liver System. The system comprises of an extracorporeal (outside the body) loop that helps process continuously a patient's whole blood, maintaining temperature, oxygenating to arterial levels, adjusting pH to 7.2 and perfusing a hollow fiber bioreactor, charged with primary porcine hepatocytes, before returning the blood to the patient.[36] The bioreactor was patented (5,955,353) in the USA in 1999. The patent describes a platform technology of high-density cell culture, that can be extended beyond liver cells, to a wide variety of other cell types including, pancreatic islets (biocritical pancreas) and other endocrine cells. The company's bioartificial liver system has also been designated as an "Orphan Product" by the US/FDA, for the treatment of acute liver failure. Phase-1 testing of the system was done at the University of Pittsburgh (http://www.upmc.edu). A news release from the University of Pittsburgh Medical Center states, "Researchers at the University of Pittsburgh Medical Center (UPMC) have begun testing a new bioartificial liver

assist system designed by Excorp Medical, Inc., that uses healthy liver cells from pigs as a means to improve the liver function of critically ill patients with liver failure. The trial is intended to assess the safety of the system, but researchers also will be paying close attention to, whether it can improve a patient's condition until transplantation is feasible, or if it can obviate the need for transplantation altogether, if the failing liver recovers." Despite the success of the testing and the FDA approval as an orphan device, the system is still not available for commercial use.

We started this article with the news about the announcement of one of the largest "Genomic" study by the NIH. Despite the fact, that DNA sequence and analysis of metabolome has become easier and less expensive, interpretation of the data developed by such studies poses a great challenge. In a recent issue of JAMA (September 25, 2018), Burke discusses a case in point in which, "All protein-coding regions of the genome (an exome analysis) in 50726 individuals, found a median of more than 20,000 gene variants per person, most of them rare, and hundreds not previously identified." According to these researchers, the evidence for most variants is limited regarding pathogenicity. Just to illustrate this point further, I will discuss yet another study, which relates to the analysis of platelet lipidome. Researchers at Cardiff University, UK, found that resting platelets have over 5600 unique lipid species with only 50% being identified.[37] In reality, a only a handful of these lipid species have been shown to play a major role in platelet physiology and function. In a review article on this topic, Dr. Steve Watson and associates state that "applications of lipidomics to platelet biology is still in its infancy, seminal studies have shaped our knowledge of how lipids regulate key aspects of platelet aggregation, shape change, coagulation and degranulation, as well as how lipids generated by platelets influence other cells, such as vascular wall, and thus how they regulate hemostasis, vascular integrity, inflammation, thrombosis and atherosclerosis." Much of this information was available prior to any lipidomic studies. The thousands of lipid species discovered by lipidomics are like "orphan molecules" begging for explanation for their role, in the sequence of events described by these authors.[38]

To end this overview on medical innovations, we would like to include the top ten medical innovations for 2018, according to the prestigious Cleveland Clinic, USA. 1) Hybrid closed-loop insulin delivery system, 2) Neuromodulation to treat obstructive sleep apnea, 3) Gene therapy for inherited retinal diseases, 4) The unprecedented reduction of LDL cholesterol, 5) The emergence of distance health, 6) Next generation vaccines, 7) Arsenal of targeted breast cancer therapies, 8) Enhanced recovery after surgery, 9) Centralized monitoring of hospital patients, 10) Scalp cooling for reducing chemo therapy induced hair loss. In addition to these well recognized innovations, advances made in the area of polymer chemistry, material sciences, have provided us an important new class of mechanical and bioprosthesis heart valves. Five-year clinical studies have been completed, on self-expanding bioprosthesis. Rapid advances in human brain-computer interface technologies, have provided an electroencephalogram-based, brain-computer interface and lower-limb prosthesis control.[39] As is the case in all other innovative areas this new area of exploration, brain-computer-interface, has made impressive achievements over the past few-years.[39-42]

Conclusion

Advances in biomedical research, as well as technology innovations, offer new hopes and transformative opportunities, for improved healthcare. Since the early discoveries of DNA/RNA, micro RNAs, and beginnings of the Human Genome Project, there is a great expectation in the medical community, as well as patient population, that there will be rapid developments in the way the healthcare is delivered. There were lots of hopes and speculation, that we will find easy solutions, to address common chronic health issues. Rapid advances in the various genetic studies, elucidating the structures of DNA, RNA, and the total genome analysis, contributed significantly to our understanding of functional role of genes, gene expressions, gene x gene, gene-environment interactions, genomics, metabolomics, role of microbiota, use of CRISP technology, gene editing, gene therapy, cellular and molecular therapies. Medical technology innovations also have made rapid progress and complemented the advances in biomedical research and innovations.

The success of biomedical research in the early 50s of polio vaccination, antibiotics, antipsychotic drugs, and equally dramatic success in the applications of cardiopulmonary bypass, dialysis, and organ transplantations, prompted financing of research both by the industry as well as the government. In a review of this topic in the N Engl J Med. The authors make a very important observation, which summarizes the collective view of the expectant individuals, "Despite the justified scientific excitement about using knowledge of the genome as a fundamental exploratory tool, unrealistic expectations for a quick route to clinical applications have produced disappointment, especially among disease groups, and companies.[43] They further emphasize that with few exceptions, new scientific discoveries require 15 to 25 years for their clinical application. Advanced countries have sponsored and supported the research initiatives using a variety of models, encouraging alliances between the Academia and the Industries, multidisciplinary approaches, multicountry investigations, establishing specialized centers of excellence (Stem Cell Institute, Imaging Institutes, Genomic Centers), Clinical and Translational Institutes. Moses and associates reviewed 70 such alliances from the mid 1960s through 2000. In their opinion, these alliances have not accelerated the pace of either discovery or clinical application. According to these researchers, source of difficulty is idiosyncratic, but recurrent problems, or a failure at inception to agree on intellectual-property provisions, excessive secrecy, and disagreements over the overall research aims.[43]

Collaborators from the Johns Hopkins School of Medicine and the Harvard Medical School propose a seven point recommendation: 1) improve data on clinical value (develop more robust analytical techniques), 2) change the role of teaching hospitals (improve ability to do early-stage-clinical studies), 3) develop new models for collaboration and financing, establish biomedical innovation trusts (support

research on high-priority diseases), 4) create new class of bonds, use incentives to promote pluralism (preference in funding might be to new institutions and for new ideas), 5) defer patents to later in the discovery chain, renew professional commitments (remove personal bias and personal incentives), 6) focus on cost-effective targets, adopt realistic research goals (embrace new realism about the difficulty of the scientific process), 7) redefine the terms of conflict (not everyone believes biomedical research is essential). These suggestions are worth considering.[43] In this overview, we have discussed some of the major news worthy discoveries like, Framingham Heart Study (discovery of modifiable risk factors for heart disease), herald of the end or reduction in the CVD deaths, innovations in tissue and cellular engineering, development bioartificial heart and 3D printed hearts, and claims that a beating heart will be available in months or years for human transplantation, bio absorbable vascular grafts as substitutes for coronary stents, gene therapies, cancer antigen receptor therapies (CAR-T), and the progress made in bioprosthesis and computer-brain-organ-system interfaces. Despite rapid progress in biomedical research and emerging technologies, availability of new products for immediate clinical applications are limited. In spite of this observed slow pace, the overall contribution of the innovations in these areas to improved healthcare is phenomenal.

Conflicts of interest

No potential conflict of interest was reported by the author.

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ORIGINAL ARTICLE

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Assessment of Association of rs2200733 SNP on Chromosome 4q25 with the Risk of the Development of Atrial Fibrillation in the Russian Population

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Abstract

The aim of our case-control study was to investigate the possible genetic association of the rs2200733 SNP on chromosome 4q25 with atrial fibrillation (AF) in the Russian population as this association has not been examined before in this ethnicity.

Methods and Results: A total of 76 unrelated individuals diagnosed with AF and 73 control subjects without any cardiovascular pathology were included in this study. The diagnosis of AF was based on ECG and/or Holter ECG data following standard diagnostic criteria. We found that the TT genotype of the rs2200733 SNP was associated with a higher risk of AF (OR=1.4, 95% CI: 1.1-12.4). The homozygote minor rare allele genotype TT of the rs2200733 SNP tended to elevate the risk of lone AF development (OR=2.5, 95% CI: 1.2-19.5). A risk of secondary AF development did not depend on the rs2200733 SNP on chromosome 4q25 (OR=0.5, 95% CI: 0.2-1.3).

Conclusion: Our results provide additional evidence for the association between the rs2200733 (4q25) SNP on chromosome 4q25 and AF, emphasizing the need for further studies examining the role of this polymorphism in AF. (**International Journal of Biomedicine. 2018;8(4):280-283.**)

Key Words: atrial fibrillation • single nucleotide polymorphism • chromosome 4q25 • rs2200733 • odds ratio

Abbreviations

AF, atrial fibrillation; **GWASs**, genome-wide association studies; **SNP**, single nucleotide polymorphism; **Afl**, auricular flutter; **OR**, odds ratio; **CI**, confidence interval.

Introduction

Recently, a lot of emphasis has been given to personalized medicine. In this connection, there are being conducted various types of research aimed at studying the genetic predictors of different disorders in the heart rhythm and cardiac conduction system.[1,2] The advent of GWASs has provided great insight into the molecular mechanisms implicated in AF.[3-7] Detecting new genetic predictors of AF is of great importance as this heart rhythm disorder is one of the most widespread (1-2% of the population) and dangerous due to its complications.[8,9] Thus, every fifth stroke is an AF-related stroke, and the mortality among AF patients is twice as high independent on other risk factors.[8,9]

In the majority of cases, AF occurs on the background of various cardio-vascular diseases and syndromes, more often on the background of hypertension, ischemic heart disease, mitral heart disease and others. However, in one-third of cases,

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people without any pathology develop AF. In such cases, this is known as lone AF.

GWASs for AF have led to the identification of novel variants that appear to confer increased susceptibility to sporadic AF. Among these, the common variant rs2200733 on chromosome 4q25 has been strongly and independently associated with an increased risk of AF in various ethnicities.[7,10-18]

The identified gene nearest rs2200733 SNP is *PITX2*, which encodes the transcription factor Pitx2c (paired-like homeodomain transcription factor 2, isoform c). While the functional implications of 4q25 variants are poorly understood, the proximity of the locus to *PITX2* presents an intriguing potential pathophysiological link to AF.[19] Reduced expression of Pitx2c, a key regulator of left-right asymmetry, has recently been linked to atrial fibrillation.[20]

Heterozygous deletion of Pitx2c, the cardiac isoform of Pitx2, in mice is sufficient to provoke increased inducibility of atrial fibrillation without obvious structural cardiac alterations,[21,22] associated with a shortening of the left atrial action potential duration.[21] There is a marked chamber specificity of Pitx2c expression in the adult heart: mRNA transcripts are expressed almost 100-fold higher in the left as compared to the right adult human and murine atrium.[21]

P. Kahr et al. revealed systematic differences between left and right atrial gene expression and supports the hypothesis that Pitx2c has a functional role in maintaining "leftness" in the atrium in adult murine and human hearts. [20] M.J. Kolek et al. found that a common 4q25 AF susceptibility allele (rs2200733) is associated with PR interval prolongation in patients with lone and typical AF and controls with no AF. Given that prolonged PR interval is an established risk factor for AF, this observation, in the context of previously described functional effects of *PITX2* deficiency, provides further knowledge about the pathophysiological link of 4q25 variants with AF.[20]

L. Shi et al.[18] carried out case-control association studies with 383 AF patients versus 851 non-AF controls and 811 ischemic stroke patients versus 688 non-stroke controls to assess the association between rs2200733 and AF as well as that between rs2200733 and ischemic stroke in a mainland Chinese Han population. Highly significant association was detected between rs2200733 and AF in a Chinese Han population (allelic $P=3.7\times10^{(-11)}$ with OR=1.81; genotypic $P=4.1\times10^{(-12)}$ with a dominant model). Moreover, significantly stronger association was found with lone AF (OR=2.40, $P=1.3\times10^{(-9)}$ compared to OR=1.59, $P=6.2\times10^{(-7)}$ for other types of AF; $P=0.02$ for two ORs).

K. Lee et al. found that the Taiwanese with the CC genotype of the rs2200733 SNP remained recessively associated with a lower risk of developing AF than those with the TT genotype (OR=0.27, 95% CI: 0.11-0.65; $P<0.01$).[16]

To test the polymorphisms on chromosome 4q25, 16q22 and 1q21 in a group of patients (pts) that underwent catheter ablation of AF, 410 patients with AF that underwent pulmonary vein isolation were included in the study performed by M. Kiliszek et al.[7] Control group (n=550) was taken from healthy population, matched for age, sex and presence of hypertension. The study showed that the T allele of rs2200733 favored the increased number of episodes of AF per month ($P=0.045$) and larger pulmonary vein diameter (recessive model, $P=0.032$) in Polish population.

Opposite results were obtained in Danish research performed by K.M. Henninqsen et al.[23] In this study, authors investigated the association of rs2200733 and lone AF in 196 young patients. Results suggested that rs220733 was not a risk factor for AF in patients with no other cardiovascular disease and with early onset of the arrhythmia.

M. Olesen et al.[6] investigated 8 SNPs in 209 patients with early-onset lone AF and 534 individuals free of AF. They found that three SNPs, rs2200733 (4q25), rs3807989 (7p31), and rs11047543 (12p12), were associated with early-onset lone AF (OR=1.62, 95% CI: 1.16-2.27; $P=0.004$ for rs2200733).

A. Ferran et al.[11] analyzed the association between two genetic variants (rs2200733 and rs7193343) in a Spanish population and the risk of developing atrial fibrillation. A case-control study included 257 case patients with AF and 379 controls. rs2200733 SNP was associated with a higher risk of AF (OR=1.87, 95% CI: 1.30-2.70).

J. Roberts et al.[24] sought to characterize the association between rs2200733 and prevalent Afl (atrial flutter) and to determine if the variant could predict AF after cavotricuspid isthmus ablation. Authors performed a genetic association study of 295 patients with Afl and/or AF and 469 controls using multivariable logistic regression. The rs2200733 rare allele was associated with an adjusted 2.06-fold increased odds of isolated Afl (95% CI: 1.13-3.76; $P=0.019$) and an adjusted 2.79-fold increased odds of a combined phenotype of AF and Afl (95% CI: 1.81-4.28; $P<0.001$).

In the study performed by K. Kalinderi et al.[17], the T/T genotype and the T allele of the rs2200733 SNP were detected more frequently in patients with AF compared to controls (13.2% vs. 2.3%, $P=0.001$, and 29.6% vs. 17.9%, $P=0.001$), suggesting that the rs2200733 SNP increases susceptibility to AF in the Greek population.

In the study performed by A. Bhanushali et al.,[25] the rs2200733 T allele was associated with the risk of lone AF (OR=2.80, 95% CI: 1.08-7.24; $P=0.042$). F. Chen et al.[26] demonstrated that rs2200733 was strongly associated with AF recurrence after ablation ($P=0.011$) and the minor allele T increased the risk for recurrence (OR=1.715). The patients with genotype TT had larger size of right atrium and superior pulmonary veins than those of CC genotype.

Data regarding a connection between the rs2200733 SNP and AF in different populations are contradictory: Some researchers show a statistically significant association with AF, in particular lone AF, whereas other studies have not found this association.

The aim of our case-control study was to investigate the possible genetic association of the rs2200733 SNP on chromosome 4q25 with AF in the Russian population as this association has not been examined before in this ethnicity.

Materials and Methods

A total of 76 unrelated individuals (41 men and 35 women) diagnosed with AF and 73 control subjects (38 men

and 35 women) without any cardiovascular pathology were included in this study. The diagnosis of AF was based on ECG and/or Holter ECG data following standard diagnostic criteria. A paroxysmal form of AF was diagnosed in 82.9% of patients, and a permanent form of AF was diagnosed in 17.1% of patients.

All patients were divided into two groups: Group 1 comprised 33 (43.4%) patients diagnosed with lone AF, Group 2 – 43 (56.6%) patients whose principal disease was hypertension (37.2%), coronary heart disease (Class II-III angina pectoris (44.2%)) or both diseases (18.6%).

The median age in the groups did not differ significantly (52 years [44.0; 63.0] and 52 years [45.5; 63.5], respectively).

All patients underwent the following examinations: ECG, echocardiography, Holter ECG, exercise stress test, transesophageal stimulation of the left atrium, and blood test for thyroid hormones. All participants were genotyped for the presence of the rs2200733 SNP using real-time polymerase chain reaction.

The present study was approved by the local Ethics Committee of Professor V.F. Voino-Yasenetsky Krasnoyarsk State Medical University. Written informed consent was obtained from each patient.

Statistical analysis was performed using SPSS v. 20.0 (SPSS Inc, Chicago, IL). For descriptive analysis, results are presented as median (Me) and interquartile range (IQR; 25th to 75th percentiles). Deviation from Hardy-Weinberg equilibrium and differences in allele distributions between the two groups were assessed by χ2- test with 1 degree of freedom (df), whereas differences in genotype distributions between cases and controls were assessed by the χ^2- test with 2 df. The odds ratio (OR) and their 95 % confidence intervals (CI) were calculated to estimate the strength of the association. For all tests, a probability value of $P<0.05$ was considered statistically significant.

Results and Discussion

We found that the TT genotype of the rs2200733 SNP was associated with a higher risk of AF (OR=1.4, 95% CI: 1.1-12.4) (Fig.1).

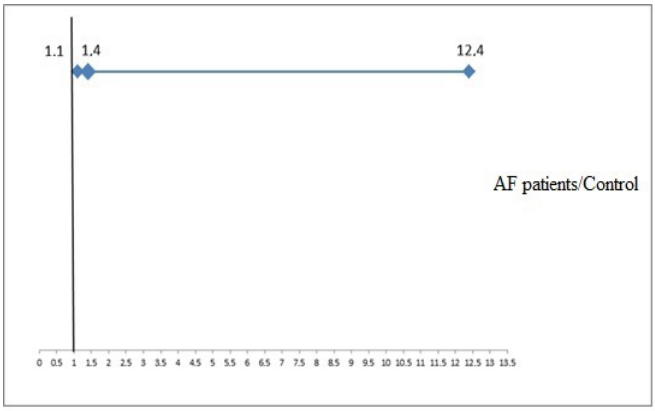

Fig. 1. *OR for the rs2200733 SNP genotype frequencies in AF patients (CC+CT relative to TT).*

The homozygote minor rare allele genotype TT of the rs2200733 SNP tended to elevate the risk of lone AF development (OR=2.5, 95% CI: 1.2-19.5) (Fig. 2). In Figures 1 and 2, we can see that 95%CI does not include value 1 (y-axis), which gives evidence that the revealed link is statistically significant.

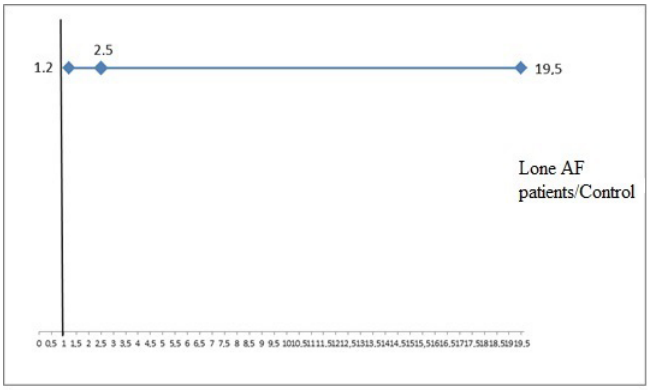

Fig. 2. *OR for the rs2200733 SNP genotype frequencies (CC+CT relative to TT) in patients with lone AF.*

A risk of secondary AF development did not depend on the rs2200733 SNP on chromosome 4q25 (OR=0.5, 95% CI: 0.2-1.3) (Fig. 3).

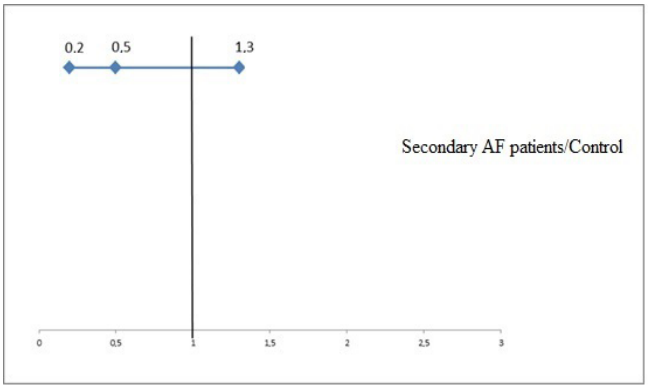

Fig. 3. *OR for the rs2200733 SNP genotype frequencies (CC+CT relative to TT) in patients with secondary AF.*

The present study showed that a TT genotype of the rs2200733 SNP on chromosome 4q25 was strongly associated with the risk of lone AF (OR=2.5, 95% CI: 1.2-19.5). The results of our research, obtained on the Russian population for the first time, are similar to those obtained earlier by a number of authors. Thus, L. Shi et al. found a stronger association of TT genotype of the rs2200733 SNP with lone AF (OR=2.40, $P=1.3\times10^{-9}$).[18] M. Olesen et al. found that the rs2200733 SNP was associated with early-onset lone AF (OR=1.62, 95% CI: 1.16-2.27; $P=0.004$).[6] J. Roberts et al. revealed that the rare T allele was associated with an adjusted 2.06-fold increased odds of isolated Afl (95% CI: 1.13-3.76, $P=0.019$) and an adjusted 2.79-fold increased odds of a combined phenotype of AF and Afl (95% CI: 1.81-4.28, $P<0.001$).[24] F. Chen et al. demonstrated that rs2200733 was strongly associated with

AF recurrence after ablation (P=0.011) and the minor allele T increased the risk for recurrence (OR=1.715).[26]

Thus, the results obtained are in line with reports of previous studies carried out in different European and Asian populations. Our results provide additional evidence for the association between the rs2200733 (4q25) SNP on chromosome 4q25 and AF, emphasizing the need for further studies examining the role of this polymorphism in AF.

Conflict of interest

The authors declare that they have no competing interests.

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A Multiple Logistic Regression Model as an Additional Mathematical Method for Predicting the Development of Ischemic Stroke in Patients with Atrial Fibrillation

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Abstract

Prevention of thromboembolic complications in cases of atrial fibrillation (AF) and, above all, ischemic stroke (IS), represents a key problem of modern cardiology. ***The aim*** of the present study was to assess the feasibility of Multiple Logistic Regression Analysis in predicting the occurrence of IS in AF patients with the predictor genotypes of the *FGB*, *GPIα*, and *GPIβα* genes, in order to implement an approach to primary prevention and personalized treatment.

Methods and Results: We examined 43 patients with atrial fibrillation and IS in their histories and 78 patients with AF without IS. A total of 188 persons without AF were included in the control group. The present study showed that the homozygote minor allele genotype (AA) of the *FGB* -455G/A SNP, the minor allele CT and TT genotypes of the *GPIa* 807C/T SNP, and the -5C/-5C and -5C/-5T genotypes of the *GPIβα* -5T/C polymorphism can be studied as genetic predictors of IS in AF patients. Logistic regression analysis was used to predict the development of IS in AF patients, depending on the presence of pathological genotypes of the *FGB*, *GPIα*, and *GPIβα* genes. The percentage of correct predictions for the absence of IS using this model was 99.5%. The development of IS was correctly predicted in 7.0% of cases. The overall percentage of correct predictions was 82.3%.

Conclusion: The obtained logistic regression model is recommended as an additional method for assessing the risk of IS in young patients with lone AF. (**International Journal of Biomedicine. 2018;8(4):284-287.**)

Key Words: atrial fibrillation • ischemic stroke • multiple logistic regression analysis • predictor genotypes

Abbreviations

AF, atrial fibrillation; **ACA**, acute cerebrovascular accident; **AUC**, area under the curve; **FGB**, fibrinogen beta chain; **IS**, ischemic stroke; **MLRA**, Multiple Logistic Regression Analysis; **OR**, odds ratio; **CI**, confidence interval; **SNP**, single nucleotide polymorphism; **GPIa**, glycoprotein Ia; **GP1βα**, glycoprotein Ib platelet subunit alpha.

Introduction

Prevention of thromboembolic complications in cases of AF and, above all, IS, represents a key problem of modern cardiology.[1-7] The formation of AF-related blood clots in the atria is a result of a complex interaction among different factors, including the enlargement of the left atrial appendage, hemostasis, endothelial dysfunction, systemic and, conceivably, local hypercoagulation.[8] The CHA2DS2-VASc score for Stroke Risk Assessment in AF presents quantitative estimates of the various clinical risk factors.[9,10] However, over the past decade a number of studies have discussed the genetic risk factors for IS development in patients with AF.[11-15] Mainly, the objects of the study were the genes of the

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hemostasis system, but more generally, the objects of study were the genes of the hemostatic system.

Several polymorphisms of platelet membrane glycoprotein have been identified as potential risk factors for cardiovascular disease. Thus, a nucleotide −5T/C dimorphism in the translation initiation site (Kozak sequence) of the GPIbα gene has been associated with increased platelet surface levels of the GPIb-IX-V receptor complex.[16] The role of this GPIbα Kozak sequence polymorphism in the occurrence of arterial thrombotic disease is known.

Platelet *GPIa* 807C/T is the only GP polymorphism associated with the expression levels of GP Ia/IIa (the platelet collagen receptor). Logistic regression analysis revealed that the presence of the *GPIa* 807C/T C allele and CC genotype were both associated with a decreased risk of CH compared with T allele, CT and TT genotypes.[17]

Xiaofeng Hu et al. found that the *FGB* -455 G/A polymorphism was independently associated with increased risk of cardioembolic stroke in AF patients with a low CHA_2DS_2-VASc score.[18]

The researchers presented data on the association of predictor genotypes with the development of IS in AF patients.[19] At the same time, the use of mathematical methods for predicting this complication in AF patients is relevant for the isolation of high-risk groups among patients and the implementation of measures for targeted prevention and use of personalized treatment.

The aim of the present study was to assess the feasibility of MLRA in predicting the occurrence of IS in AF patients with the predictor genotypes of the *FGB*, *GPIα*, and *GPIβα* genes, in order to implement an approach to primary prevention and personalized treatment.

Materials and Methods

We examined 43 patients with AF and IS in their histories (Group 1) and 78 patients with AF without IS (Group 2). A total of 188 persons without AF were included in the control group (Group 3). The median age in the groups did not differ significantly (58 years [52; 65], 62 years [44.75; 71], and 59 years [53; 65.75], respectively). The diagnosis of AF was based on electrocardiograms (ECG) and/or Holter ECG data following standard diagnostic criteria. All patients underwent the following examinations: ECG, echocardiography, Holter ECG, exercise stress test, transesophageal stimulation of the left atrium, and blood test for thyroid hormones (TSH, free T3, free T4). To confirm the ischemic nature of acute cerebrovascular accident in the examined patients, a brain MRI was used. All participants underwent a molecular-genetic examination.

The present study was approved by the local Ethics Committee of Professor V.F. Voino-Yasenetsky Krasnoyarsk State Medical University. Written informed consent was obtained from each patient.

Statistical analysis was performed using the Statistica 7.0 software package (Stat-Soft Inc., USA) and IBM SPSS 20 (*SPSS* Inc, Chicago, IL). The normality of distribution of continuous variables was tested by the Shapiro-Wilk test. For descriptive analysis, results are presented as mean±standard deviation, median, interquartile range (IQR; 25th to 75th percentiles). Chi-squared and Fisher's exact tests were used to determine the association between categorical measure including allele and genotype. Three exact probability tests for departure from HWE due to heterozygote excess, heterozygote deficit and omnibus probability test were carried out using GENEPOP (v.4.7.0). The strength of the associations was expressed as odds ratio (OR) with 95% confidence interval (CI). Logistic regression analysis was used to predict the development of IS in AF patients, depending on the presence of pathological genotypes of the *FGB*, *GPIα*, and *GPIβα* genes. As a response, a binary variable was considered, where *0* is the absence of the predicted state (IS), *1* - its presence. The quality of the model (specificity and sensitivity) was measured by receiver operating characteristic (ROC) analysis. For all tests, a probability value of $P<0.05$ was considered statistically significant.

Results

The homozygote minor allele genotype (AA) of the *FGB* -455G/A SNP tended to elevate the risk of IS (OR=1.7, 95% CI: 1.08–2.82) in AF patients. Both minor allele TT and CT genotypes of the *GPIa* 807C/T SNP were associated with an increased risk of IS in AF patients (OR=2.5, 95% CI: 1.17-5.36). Analysis of −5T/C polymorphism of the *GPIbα* gene revealed that the rare -5C allele tended to elevate the risk of IS (OR=1.9, 95% CI: 1.05–3.42), and both -5C/-5C and -5C/-5T genotypes were associated with an increased risk of IS in AF patients (OR=2.3, 95% CI: 1.15–4.57).

Thus, the present study showed that the homozygote minor allele genotype (AA) of the *FGB* -455G/A SNP, the minor allele CT and TT genotypes of the *GPIa* 807C/T SNP, and the -5C/-5C and -5C/-5T genotypes of the *GPIβα* −5T/C polymorphism can be studied as genetic predictors of IS in AF patients. Therefore, for the further development of mathematical models to predict IS, depending on the predictor genotypes in AF patients, we used the AA genotype of the *FGB* -455G/A SNP, the TT+CT genotypes of the *GPIa* 807C/T SNP, and the -5C/-5C+-5C/-5T genotypes of the *GPIβα* gene.

The logistic regression model is a dependence of the logarithm of the chance of the predicted event (logit) on a linear combination of factor variables. Accordingly, the probability that the predicted event will occur can be represented by the following equation: $p = \frac{1}{1 + e^{-(b_0 + b_1 x_1 + \ldots + b_n x_n)}}$, where

p - the probability of the predicted event,

e - the mathematical constant 2.72 (the base of the natural logarithms),

b_0 - the constant of the model, b_1 is the coefficient of the predictor variable

x_1, - the change in logarithmic chances caused by a single change in independent variables,

n - the ordinal number of the predictor included in the equation.

When predictors were included in the multiple logistic regression equation, their collinearity and autocorrelation were tested.

The logistic regression model was built using step-by-step

inclusion of prognostic factors and determination of the minimum set of predictors by calculating Nagelkerke's R^2 to indicate the effect of all model predictors on response variable dispersion.

The statistical significance of the model was verified using the $\chi 2$ criterion. At a value of $P<0.05$, the hypothesis of insignificance of the model was rejected. Compliance of the model with the data was characterized by the Hosmer-Lemeshow goodness-of-fit test (HL test). When $P>0.05$, the hypothesis of model consistency was accepted.

The interpretation of the logistic regression parameters was based on the value of *exp(b):* if the coefficient *b* is positive, then *exp(b)* is greater than 1 and the chances of the predicted event increase; if the coefficient is negative, the chances decrease.

Sensitivity and specificity of predictors were evaluated by ROC-analysis. Quantitative interpretation of the results for the final model was carried out on the ROC-curve with the assessment of AUC.

To assess the impact of these genotypes on the probability of IS, a multiple logistic regression model was constructed:

$$p = \frac{1}{1 + e^{-(-2{,}519 + 0{,}94x_1 + 0{,}927x_2 + 0{,}854x_3)}}$$, where

p - the probability of ischemic stroke

x1- presence of the AA genotype of the *FGB* -455G/A SNP (yes – 1, no – 0), (b_1= 0.94)

x2 - presence of allele T (TT or CT genotypes) of the *GPIa* 807C/T SNP (yes – 1, no – 0), (b_2= 0.927)

x3 - presence of allele -5C (-5C/-5C or -5C/-5T genotypes) of the *GPIβα* −5T/C polymorphism (yes – 1, no-0), (b_3= 0,854),

constant b_0 = - 2,519.

The model was statistically significant. The significance of the model at the third step of the predictors' inclusion corresponded to P=0.002. The HL test demonstrates the consistency of the model with the original data (P=0.054). The data of the model is presented in Table 1.

The model reflects an increase in the possibility of IS in AF under the influence of 3 studied genetic factors. The coefficient of determination of the multiple model is R^2=0.102, which shows a statistically significant explanation of the influence of selected genetic factors on the probability of IS in the study group of patients by 10.2%. The obtained model has a high specificity. The percentage of correct predictions for the absence of IS using this model was 99.5%. The development of IS was correctly predicted in 7.0% of cases. The overall percentage of correct predictions was 82.3%.

According to the ROC curve analysis, AUC was 0.671±0.050 (95% CI: 0.574-0.768; P<0.001), which corresponds to the average quality of the model for predicting the development of IS in AF (Fig.1).

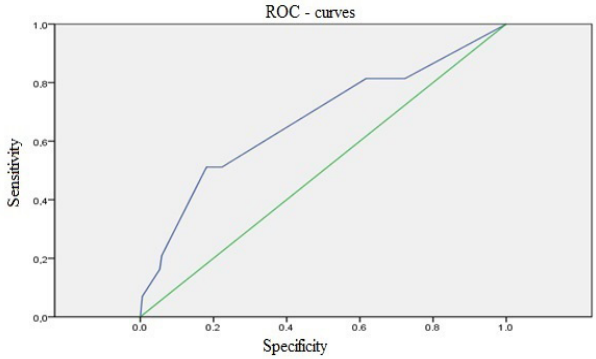

Fig. 1. *ROC - curve of the regression model of IS in AF patients depending on the presence of the predictor genotypes.*

According to the overall results of our study, the probability of IS in AF, predicted by the method of logical regression, is 7.0%; for the absence of IS - 99.5%. The total percentage of correct predictions is 82.3%.

Table 1. Linear regression model of IS depending on the presence of pathological genotypes

		Regression coefficient (b)	Standar d Error	Wald test χ^2	Sig. (P)	Exp (b)	95% CI for Exp(b)	
							Lower Bound	Upper Bound
Step 1	*GPIα* gene (TT and CT genotypes)	0.916	0.390	5.515	0.019	2.498	1.163	5.364
	Constant	-2.092	0.335	38.950	<0.001	0.123		
Step 2	*GPIα* gene (TT and CT genotypes)	0.906	0.394	5.293	0.021	2.474	1.144	5.350
	GPIα gene (CC and CT genotypes)	0.817	0.358	5.194	0.023	2.263	1.121	4.568
	Constant	-2.350	0.365	41.533	<0.001	0.095		
Step 3	*FGB* gene (AA genotype)	0.940	0.463	4.125	0.042	2.560	1.033	6.344
	GPIβα gene (-5T/-5T and -5C/-5T genotypes)	0.927	0.398	5.423	0.020	2.526	1.158	5.510
	GPIβα gene (-5C/-5C and -5C/-5T genotypes)	0.854	0.363	5.528	0.019	2.348	1.153	4.785
	Constant	-2.519	0.384	43.019	<0.001	0.081		

Discussion

Recently, a lot of emphasis has been given to personalized prophylaxis, which is aimed at managing the genetic risk factor. New knowledge about genetic risk factors from population-based genetic cohort studies requires the creation of different data analytics software to discover the ratio of genetic risk factors and the influence of environmental factors, as well as the impact of comorbidity. In particular, the developed logistic regression model represents an important method of analysis, which is used to confirm the statistical significance of the chosen genetic predictors of IS development in AF patients. The described model can be used as an additional method of predicting the AF-related IS.

Using the developed logistic regression model of IS prediction, depending on predictor genotypes in AF patients, it is possible to take into account genetic factors to improve the complex of preventive measures for a particular individual, this being very important at a low risk of AF-related embolic complications, according to the CHA2DS2-VASc score. The obtained logistic regression model is recommended as an additional method for assessing the risk of IS in young patients with lone AF. To increase the sensitivity of the developed multiple logistic regression model and obtain a more accurate forecast, it is possible to further include in the model the main clinical risk factors for AF-related IS used in the CHA2DS2-VASc score.

Conflict of interest

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Cardiology

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Synchronization of Wave Flows of Arterial and Venous Blood and Phases of the Cardiac Cycle: Part 3

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Abstract

Hemodynamic indices studied in healthy people were obtained by catheterization in various vascular areas: the chambers of the heart (ventricles, atria, coronary sinus), pulmonary trunk, sigmoid sinus, aorta, inferior vena cava, superior vena cava (SVC), and right hepatic vein (RHV). Using the mean values of the hemodynamic parameters, we constructed graphics of the curves of the central, arterial, and venous pressure, synchronized with each other, with an ECG, and with pulse wave.

The complex of hemodynamic curves, supplemented by curves of RHV and SVC, revealed a coincidence in one zone of temporal equalization of pressure (ZTEP) for values of the left atrium, coronary sinus, right ventricle, sigmoid sinus, SVC, and RHV(wedged). We identified this ZTEP as a hemodynamic trigger (T-point), assuming that this point is the trigger point for the launch of the high-energy processes of the right heart, forming the following cardiac cycle: the beginning of the systole of right ventricle, the opening of pulmonary valve. (**International Journal of Biomedicine. 2018;8(4):288-291.**)

Key Words: cardiac cycle • hemodynamic parameters • ECG • wave flows

Abbreviations

Ao, aorta; **AV**, aortic valve; **CC**, cardiac cycle; **CS**, coronary sinus; **CNS**, central nervous system; **DP**, diastolic pressure; **IVC**, inferior vena cava; **IHH**, intrahepatic hemodynamics; **LA**, left atrium; **LV**, left ventricle; **MV**, mitral valve; **PC**, pulmonary circulation; **PT**, pulmonary trunk; **Pvs**, pulmonary veins; **PV**, pulmonary valve; **PW**, pulse wave; **RV**, right ventricle; **RA**, right atrium; **RHV**, right hepatic vein; **SC**, systemic circulation; **SP**, systolic pressure **SS**, sigmoid sinus; **SVC**, superior vena cava; **TV**, tricuspid valve; **ZTEP**, zone of temporal equalization of pressure.

Basic Part

The purpose of this part of the work was to study the hemodynamic processes of RA, as a single hydrohemodynamic formation, including SVC and the mouth of RHV.

Methods and Discussion

In addition to the hemodynamic data given in previous articles,[1-4] Table of Norms (Table 1) includes graphical curves: SVC and RHV(wedged, i.e., IHH).

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The hemodynamic contour of SVC almost completely repeats the hemodynamic contour of RA, making up a single hydrodynamic cavity with it (note the smaller dependence of the RA cavity on external factors: for example, breathing). The recording of hemodynamic curves during catheterization was carried out with the subject in the supine position (minimizing the effect of the gravity vector on the capacitive vessels), with holding the breath on the exhale (minimizing the dynamics of intrathoracic pressure on the capacitive vessels).

RHV(wedged), which we included in the graphic complex, demonstrates the dynamics of pressure in the venous part of the microcirculation vessels of the liver: IHHs.p. – transmission pressure from Ao through the a.hepatica system; IHHd.p. – a pressure in the microcirculation system of the

liver after a vascular response of arterioles to PW (according to the Ostroumov-Beilis reaction); straight sections of the graph reflect the pressure in the microcirculation system caused by pressure in the v.portae. Thus, the graphic representation of IHH, in our opinion, reflects a number of functions that form the microcirculatory regulation of the liver, with the participation of both a.hepatica and v.portae. The combination of the CS impulse at the onset of RA systole and the suction effect of the systole itself stimulate the opening of outflow vessels from the liver and the inflow into it from v.portae. The myogenic response of arterioles restricts the a.hepatica inflow (and, we also assume, the v.portae inflow), which maintains a constant level of pressure in the liver acinus. Evidence of this is the constancy of the portal part of the graph of the IHH curve (despite the changing conditions of inflow and outflow associated with the activity of the heart), ensuring the autonomy of portal and acinus pressure, which contributes to the stabilization of biochemical processes in the liver acinus. It should be noted that the maximum number of graphic combinations in one ZTEP falls on the curves of IHH and SS, which, in our opinion, may indicate a combined effect of these zones (brain and splanchnic organs) on the formation of the cardiac cycle phases.

The coincidence of the successive spread of contractions of various parts of the heart with the course of Purkinje fibers was noted in early studies.[5] In the embryonic heart, at the stage of development of the tube, which has already begun to pulsate, the venous sinus is first contracted, then successively the rudiments of auricles, ventricles, and aortic bulb. This is a period when the heart rudiments do not receive any nerve impulses (nerve fibers have not yet grown to muscle tissue); therefore, the impulse starts inside the organ, in its tissues, in particular, in the tissues of the venous sinus, spreading throughout the heart rudiment. At later stages of development, as well as in adult organisms, it was found that the impulse to contraction arises precisely in the part developing from the venous sinus (i.e., in the place of transition of SVC into RA (sinus node) and on the right triangle of the central fibrous body (atrioventricular node). Thus, the heartbeats, starting from the embryonic period and ending with the developed heart, are autonomous, having a myogenic nature. It is this system that forms the functional integrity of the heart, with the subsequent increasing role of CNS impulses in the heart rhythm when conscious, emotionally significant, stressful situations occur.

The fact of active, uninterrupted work of the denervated heart[6,7] (i.e., in the absence of a direct connection with CNS) is evidence of the systemic autonomy of the heart and the cardiovascular system as a whole. A pulsed trigger is the electrical discharge of the sinus node, which triggers synchronized successive phases of the heart's activity, which is a hydrohemodynamic system consisting of the periodically connecting-disconnecting chambers and conducting vessels (separated by valves), the phase synchronization of which occurs according to the principle of overpressure at the inlet (into the vessel, into the chamber), with a feedback control system. Thus, a developed heart has an autonomous (biologically earlier, preceding nervous regulation) system for triggering a contraction of syncytium (having an automated nature), as well as a system of control signals from CNS, providing an adequate physiological response to environmental stimuli.

Control signals from CNS (n.vagus and sympathetic nerves) provide an adequate response of the heart (frequency, filling, etc.) to stimuli imposed by the external environment, and regulate the activity of the heart and adequate organ blood flow, providing a hemodynamic and metabolic response to environmental stimuli, if necessary (psychological image of danger, emotional stress, etc.).

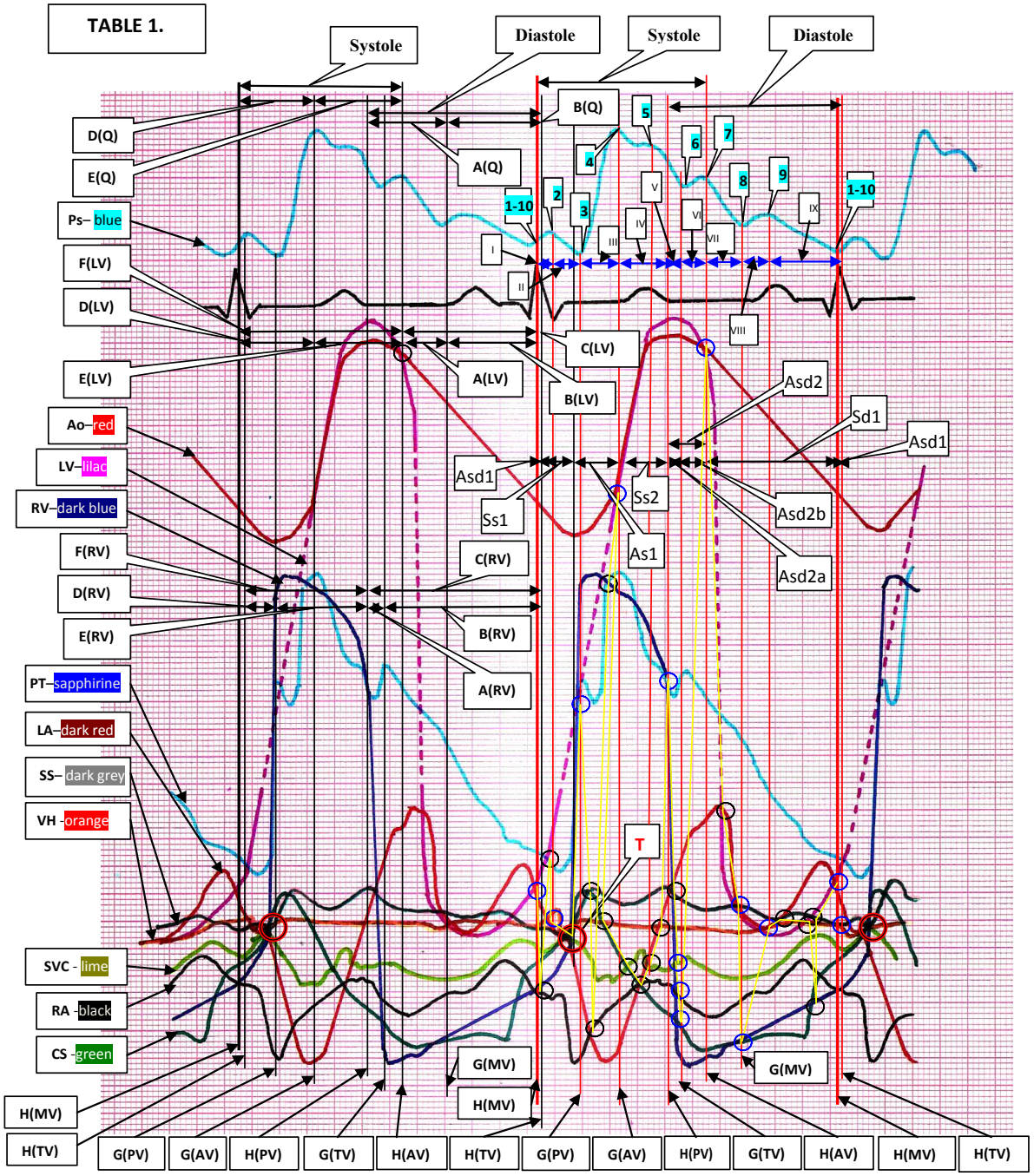
For the figurative (complementary two-dimensional graphic picture) representation of the activity of the cardiovascular system, the synchronous interaction of the totality of vessels and chambers, which we graphically show in Table 1, we describe the hydrohemodynamics of the heart as a system as a whole. In the three-chamber block of ventricles operating in a quantum generator mode, the right and left ventricles (separated by an interventricular septum) form three pairs of successively alternating arterial and venous volumetric modules of variable capacity:

A) In diastole, the atrio-ventricular module is formed, as a result of combining the three-chamber block of ventricles with the atrial block at its complete separation from the aorto-pulmonary block. At the same time, the venous volumetric modules of variable capacitance of SC (IVC-SVC-RA-CS-RV) and PC (Pvs-LA-LV) are separated from arterial volumetric modules of variable capacitance of SC(Ao) and PC(PT) by the membrane valves at the outlet of the heart (with PV for SC and AV for PC), separating them from the aorto-pulmonary block. In this phase, with open atrioventricular valves the atrio-ventricular module of the variable capacitance of SC and PC (the three-chamber block of ventricles+the atrial block) acts as a receiver of hemodynamic volumes and wave signals entering the right heart from the IVC and SVC exchange zones, and the left heart from the lung.

B) In the isometric phase of ventricular contraction with the closed heart valves, an atrial-spongy module of variable capacity is formed by connecting to the atrial block, the venous (spongy) myocardial chamber (by CS-bypass) when completely separated from the aorto-pulmonary block. At the same time, the venous volumetric modules of SC (IVC-SVC-RA-CS) and PC (PV and LA) are separated from arterial volumetric modules of SC(Ao) and PC(PT) by two membranes at the entrance and exit from the ventricular cavities (TV and PV for SC and PC, MV and valve AV for PC and SC). In this phase, with the participation of both ventricles, a CS impulse is formed and generated (in the initial phase of LV: Asd1 period), complemented by RV (Ss1 period). Unlike CS, whose impulse enters RA during formation, the spheroid of pulse wave enters the bloodstream already formed during the entire isometric ventricular contraction phase, as a result of the opening of the semilunar valves.

C) In the systole, a ventricle-aortic-pulmonary module is formed by combining the three-chamber block of ventricles with the aorto-pulmonary block (ventricular- aorto - pulmonary module), having a hemodynamic connection with the atrial block by CS (supporting pressure of right atrium as feedback).

TABLE 1.



Abbreviations

10 – **characteristic points of PW (V.V. Boronoev)**	**H(PV)**– closing of PV
A(Q) – isometric ventricular relaxation	**G(TV)**– opening of TV
B(Q) – actual ventricular diastole	**H(AV)**– closing of AV
C(LV) – LV diastole	**G(MV)**– opening of MV
A(LV)– isometric relaxation of LV	**H(TV)**– closing of TV
B(LV)– actual LV diastole	**H(MV)**– closing of MV
C(RV)– RV diastole	**G(PV)**– opening of PV
A(RV)– isometric relaxation of RV	**H(PV)** – closing of PV
B(RV)– actual RV diastole	**Asd1**– asynchronous period of ventricular systole-diastole -1
D(Q)– isometric ventricular contraction	**Ss1**– synchronization period of isometric ventricular contraction-1
E(Q)– actual ventricular systole	**As1**– asynchronous period of ventricular systole -1
F(LV)– LV systole	**Ss2**– synchronization of the actual ventricular systole -2
D(LV)– LV isometric ventricular contraction	**Asd2**– asynchronous period of ventricular systole-diastole -2
E(LV)– actual LV systole	**Ad1**– asynchronous period of ventricular diastole -1
F(RV)– RV systole	**Sd1**– period of synchronization of ventricular diastole-1
D(RV)– RV isometric contraction period	- dynamic curve ZTEP
E(RV)– actual RV systole	↔ – PW phases and ○ – coincidence between ZTEP and PW points
G(AV)– opening of AV	○ – T-point

At the same time, the venous volumetric modules of SC (IVC-SVC-RA-CS) and PC (Pvs-LA) are separated from the arterial volumetric modules of SC (LV-Ao) and PC (RV-PT) by a single membrane at the entrance to the three-chamber block of ventricles (TV for SC and MV for PC).

We believe that the described change of temporary arterial and venous volumetric modules of variable capacity is carried out due to the movement of hemodynamic volumes by the myocardium and the synchronized creation of excessive hydrodynamic pressure on the inflow to the valves of the heart vessels and chambers. The combination of "closing-opening" valves is implemented with the participation of fibrous filaments, which are an integral part of the fibrous skeleton of the heart, penetrating the entire myocardium. We believe that the wave impulses generated by the myocardium propagate both through incompressible blood boles and through the system of the fibrous skeleton of the heart, carrying out synchronous autoregulation of the heart, which does not depend on CNS.

We believe it is extremely important to note the coincidence of the values of hemodynamic "curves" in one ZTEP(LA-CS-SVC-RV-RHV-SS) that coincides with the opening of PV, synchronized (vertically) with the minimum pressure level in Ao. We designated this ZTEP as a hemodynamic trigger (T-point), assuming that this phase is the trigger point for the launch of the high-energy processes of the right heart, which form the following CC: the beginning of RV systole, the opening of PV (Table 1). Changes in the conditions of the body's functioning (changing the body's position relative to the gravity vector, etc.) can lead to the migration of the T-point, we believe, in the direction of lower pressure levels (i.e., in RA) that can create combinations of hemodynamic participants of ZTEPs, different from those registered by us.

In conclusion, concurrence of the values of hemodynamic curves (1. LA, 2. CS; 3. SVC; 4. RV; 5. RHV; 6. SS) in one ZTEP, synchronous with the opening of PV and the minimum pressure level in Ao, for the conditions in which human catheterization was carried out, is a trigger hemodynamic point (T-point)—launch point—of high-energy processes in the right heart, which form the following CC: the beginning of RV systole, the opening of PV. We believe that a change in the body's position relative to the gravity vector can lead to the migration of the T-point, which can create combinations of the structure of ZTEPs different from those registered by us.

Conflict of interest

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Cardiology

INTERNATIONAL JOURNAL OF BIOMEDICINE

Increasing Availability of the International Normalized Ratio Control in Russia

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Abstract

***Background*:** Warfarin is still, in some cases, the only medication to prevent thromboembolic complications. Warfarin intake imposes regular INR monitoring, which can be performed domiciliary. Currently, in the Russian market, there are two models of automatic portable blood coagulometers: CoaguChek XS (Germany) and qLabs ElectroMeter (China). The main problem of portable coagulometers is their high cost and high cost of operation, which the majority of patients cannot afford. To explore the demand for development of a Russian coagulometer with a more affordable price, a questionnaire survey was carried out among the patients who needed this device.

Methods and Results: We surveyed 70 patients taking Warfarin, with 5 years duration paroxysmal, persistent/or stable atrial fibrillation of nonvalvular etiology, having ≥2 CHADS-VASc score for thrombembolia risk assessment and ≤3 HAS-BLED score for hemorrhage risk assessment. According to the survey results, 7 (10%) patients had portable coagulometers, including 3 persons with CoaguChek XS and 4 persons with Micropoint qLabs ElectroMeter. Among these patients, there were 4 persons who continued regular INR monitoring domiciliary, while 3 patients had financial difficulties in getting testing strips. At the same time, 14 (20%) patients were not aware of the possibility of domiciliary INR monitoring. Patients who received regular INR monitoring domiciliary with a portable coagulometer, or at their local polyclinics, had neither ischemic strokes nor hemorrhages within a period of five years.

Conclusion: It is critical to develop and manufacture a domestic equivalent of a portable coagulometer and testing strips for household use at a more affordable price. (**International Journal of Biomedicine. 2018;8(4):292-295.**)

Key Words: portable coagulometers • international normalized ratio • atrial fibrillation • Warfarin

Abbreviations

INR, international normalized ratio; **PT**, prothrombin time; **TP**, thromboplastin; **ISI**, international sensitivity index.

Introduction

In spite of a wide choice of modern, indirect oral anticoagulants that do not require the INR control, Warfarin is still, in some cases, the only medication to prevent thromboembolic complications in pediatrics, in cases of atrial fibrillation of valve etiology, heart valve replacement, and clots in the formed aneurism as a result of an acute myocardial infarction.[1-3]

The history of Warfarin discovery goes back to 1920s, when in North Dakota (USA) the cattle started to die of a fatal—sometimes spontaneous—bleeding after such minor traumas as dehorning or castration. Carrying out the postmortem study of those animals, F. Schofield detected some

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sweet clover (*Melilotus alba* and *M. officinalis*) in the intestine of a dead cow. F. Schofield suggested that spoilt clover caused an unknown disease, and proved his assumption by his study on rabbits. In 1933, a group of scientists-chemists under the guidance of K.P. Link managed to obtain and identify a substance from spoilt hay that was later named *dicumarol*. In 1936, H. Dam established that the cause of an increased bleeding was that *dicumarol* brought down the liposoluble vitamin K level. Initially *dicumarol* was used as a rat poison named WARFARIN, and in 1947, it was used for the first time as a medicament to treat an acute myocardial infarction.[4,5]

The mechanism of *dicumarol* action is due to a decrease in the synthesis and functional activity of blood clotting factors (II, VII, IX, X) and the loss of their ability to bind to phospholipids. This is connected to impaired decarboxylation and carboxylation of proteins dependent on vitamin K due to inhibition of the enzyme vitamin K oxide reductase in the liver.[6]

Taking Warfarin involves regular monitoring of the extrinsic pathway of blood coagulation, because as an antagonist of vitamin K, its effectiveness changes depending on the number of products containing vitamin K and functional characteristics of the liver. A method for determining prothrombin time (PT) was introduced into practice by A.J. Quick[1,2,7] in 1935. PT is the period of time during which plasma coagulation occurs with the formation of a clot after Thromboplastin (TP) and calcium chloride are added to it. TP is a lipidized tissue factor obtained by extracting salt from tissues of mammals or by genetic engineering.[7,8] The activity of the extrinsic and common pathways of blood coagulation and characteristics and concentration of TP can influence the results of PT.[7] PT can be shown in three variants: The first one is the clotting time in seconds. The second variant shows prothrombin index. The third variant represents the prothrombin ratio: $\frac{PT\ 100\%}{PTp}$, where

PTp is PT of patient plasma (in seconds)
PT 100% is PT of pool of fresh donor plasma that contains 100% of clotting factors (in seconds).

However, it turned out that different laboratories could obtain different results of PT. Possible reasons for the variations in these indicators are the use of different TPs, and sampling and evaluation techniques. In order to standardize the test, it was decided to take into account the degree of TP activity in the form of international sensitivity index (ICI).

ICI shows the ability of TP to change the plasma clotting time depending on the deficiency of factors II, VII and X. WHO ordered producers to compare their TP with the reference, which was accepted as the unit. In 1983, the following formula of INR with ISI was accepted and put into practice: $(\frac{PT\ 100\%}{PTp})^{ISI}$

In addition to the sensitivity of TP, the results of the prothrombin test can be influenced by the equipment used to measure the time of clot formation (types of coagulometers). Therefore, to avoid inaccuracy, thromboplastin manufacturers release reagents adapted to the methods of fibrin clot registration.[7-9]

If a patient takes Warfarin regularly, frequent repeated INR evaluation is required. Over the most recent years, it has become more popular to evaluate INR in capillary blood; it makes blood sampling easier, and the volume of blood necessary for testing is smaller. Despite these obvious advantages, some difficulties arise. The major problem is in a high risk of blood contacting the thrombogenic tissue surface, which can trigger the coagulation process much sooner that would result in a preanalytical error. Another difficulty is the calibration of the proper quality control system for prothrombin test methods proposed by domestic thromboplastin producers. In these methods, whole blood and regular TP are used. Whole blood has a higher density and viscosity than plasma, which leads to a hampered blending of the reaction mixture in the blood coagulometer, and which in turn yields a faulty result. A possible way to solve this problem is by applying combined reagents whose composition, apart from TP, includes fibrinogen and V factor, which allows decreasing the capillary blood volume to 10-20µl (1/20 of the medium volume). A high percentage of the sample dilution allows using lyophilizated plasmas in the ratio of ½ of blood volume for the quality control. In this case, one can disregard the influence of blood cells (<3% of the reaction mixture). When whole blood in used, the hematocrit affects the result significantly. In this connection, a nomogram is necessary to compensate for the influence of hematocrit. The above-stated problems were solved in portable blood coagulometers by means of pre-calibrated test strips containing TP (usually TP of genetically engineered origin is used).[7,8]

Currently, in the Russian market, there are two models of automatic portable blood coagulometers that can be used by patients at home (CoaguChek XS portable coagulometer and qLabs ElectroMeter). They have similar characteristics and are easy to use (Table 1).

Table 1.

Portable coagulometers performance comparison

Specifications	CoaguChek XS	qLabs ElectroMeter
Country of origin	Germany	China
Sample type	Fresh whole capillary blood or whole venous blood	Fresh whole capillary blood
Instrumental error	Maximal instrumental error in case of capillary blood analysis - ±4.5%, in case of venous blood analysis - ±3.5%	<5%
Measurement range	0.8-8	0.5-7.5
Measurement method	Electrochemical	Electrochemical
Connection with PC	With the use of Coaguchek XS Connect device (is not a part of package)	With the use of USB cable (supplied as part of package)
Weight	127 g (without batteries)	126 g (without batteries)
Price as of August 24, 2018 from a substantive analysis of suppliers pricing	From 35,500 RUB	From 19,490 RUB
Testing strips price as of August 24, 2018 (24 pcs.) from a substantive analysis of suppliers pricing	From7,100 RUB (item - 295.83 RUB)	From 5,300 RUB (item - 220.8 RUB)

The main problem of portable coagulometers is their high cost and high cost of operation, which the majority of patients cannot afford. That is why, in many sparsely populated areas situated far from certified laboratories, INR control is still a problem. The appearance of a less expensive domestic analogue would probably help to partially solve this problem.

Materials and Methods

To explore the demand for development of a Russian coagulometer with a more affordable price, a questionnaire survey was carried out among the patients who needed this device.

We surveyed 70 patients (26 males and 44 females) taking Warfarin, with 5 years duration paroxysmal, persistent/or stable atrial fibrillation of nonvalvular etiology, having ≥2 CHADS-VASc score for thrombembolia risk assessment and ≤3 HAS-BLED score for hemorrhage risk assessment. The patients' median age was 59.00 [47.00-65.00] years. All the study participants were Krasnoyarsk city residents. The present study was approved by the local Ethics Committee of Professor V.F. Voino-Yasenetsky Krasnoyarsk State Medical University (Record No. 88 dated 06.06.2018). Written informed consent was obtained from each patient.

Before the survey, patients were informed of the possibility of performing INR domiciliary with a portable coagulometer, as well as of comparative characteristics of portable coagulometers (Table 1). A special workshop was conducted to teach the patients how to monitor INR with a portable coagulometer (CoaguChek XS or Micropoint qLabs ElectroMeter).

Results

Single INR measurement showed a therapeutic range from 2 to 3 in all patients. According to the survey results, 7 (10%) patients had portable coagulometers, including 3 persons with CoaguChek XS and 4 persons with Micropoint qLabs ElectroMeter. Among these patients, there were 4 persons who continued regular INR monitoring domiciliary, while 3 patients had financial difficulties in getting testing strips.

Further survey questions allowed us to reveal the reaons why 63 (90%) patients did not buy portable coagulometers to control their INR. Among these patients, there were 19 (30.2%) who received regular INR monitoring at their local polyclinics and 31 (49.2%) who received irregular INR monitoring at their local polyclinics. These patients wanted to buy a portable coagulometer, but could not afford it. At the same time, 13 (20.6%) patients did not realize the significance of regular INR monitoring (Figure 1).

Moreover, it should be indicated that 14 (20%) patients were not aware of the possibility of domiciliary INR monitoring. Among them, there were 4 (28.6%) patients who received regular INR monitoring at their local polyclinics and 5 (35.7%) patients who received irregular INR monitoring at their local polyclinics. These patients wanted to buy a portable coagulometer, but could not afford it, and 5 (35.7%) patients were not aware of the possibility and significance of domiciliary INR monitoring.

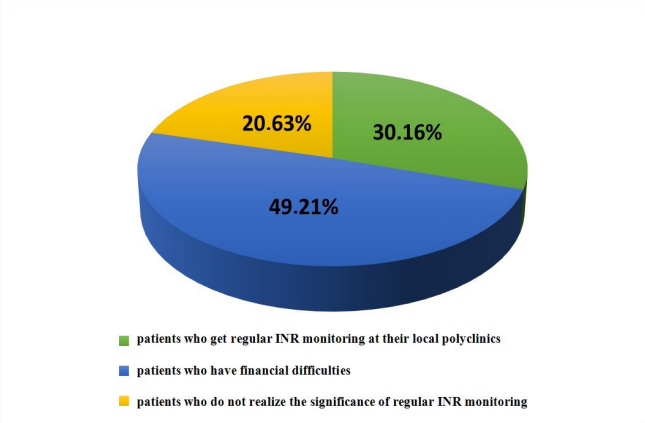

Fig. 1. *Reasons why the patients who needed regular INR monitoring did not buy portable coagulometers.*

As it turned out, those patients who received regular INR monitoring domiciliary with a portable coagulometer, or at their local polyclinics, had neither ischemic strokes nor hemorrhages within a period of five years.

Among 44 (62.8%) patients without regular INR monitoring, atrial fibrillation was complicated by ischemic stroke in 5 (11.4%) persons and by different hemorrhages in 7 (15.9%) persons (Figure 2).

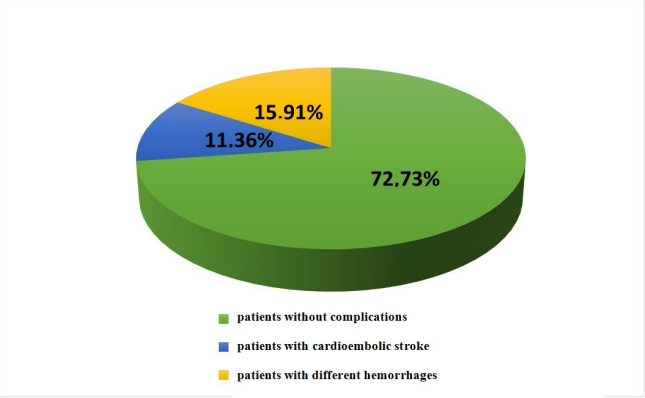

Fig. 2. *Complications in patients who did not have regular INR monitoring.*

Discussion

The results described above indicate that it is critical to develop and manufacture a domestic equivalent of a portable coagulometer and testing strips for household use at a more affordable price.

Up to now, the problem of INR control, especially for patients whose mobility is limited or those who live far from big towns and cities, is still urgent. There is still a high frequency of complications resulting from taking antagonists of vitamin K, such as bleeding and an inadequate therapeutic effect of medication due to improper INR control.[1-3] Informing patients and doctors about the possibilities provided by modern, automatic, portable blood coagulometers will allow improving

INR control; and using portable blood coagulometers by means of telemedicine and/or special apps for telephones will also allow improving INR control and decreasing the load on the medical network, resulting in lower public health expenses.[10,11]

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Conflict of interest

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Women's Health

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Sleep Apnea in Caucasian and Asian Climacteric Women

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Abstract

The aim of our study was to define the structure of sleep in Caucasian and Asian peri- and postmenopausal women with sleep apnea.

Materials and Methods: Two hundred and forty-seven menopausal women between 45 and 60 years of age participated in this study. The participants were divided into 2 ethnic groups, taking into account the genealogy (the representatives having in two generations of parents of one ethnic group) and self-identification, taking into account phenotype elements. Caucasians were represented by the Russian ethnic group (n=115) and Asians by the Buryat ethnic group (n=132). The study included the collection of anamnestic data, physical examinations, clinical (Berlin questionnaire, general medical examination, gynecological examination, polysomnographic monitoring according to American Academy of Sleep Medicine recommendations), and biochemical laboratory methods.

Results: Sleep complaints, such as difficulty falling asleep, frequent night awakenings (more than 2 times), difficulties of morning awakening or problems waking up too early, were increased in perimenopausal Caucasian women. We found ethnic differences in a greater frequency of complaints in Caucasian women, but Asian women also showed a tendency to increased complaints about poor sleep. Polysomnographic monitoring revealed that OSA was more common in Asian postmenopausal women than in Caucasians. However, in structure of sleep we have not seen distinctions.

Conclusion: For improving the quality of life, it is necessary not only to pay attention to menopausal problems, but also to solve the sleep problems associated with various strategies, both age management and lifestyle. (**International Journal of Biomedicine. 2018;8(4):296-300.**)

Key Words: climacteric women • sleep apnea • Caucasians • Asians • sleep structure

Abbreviations

BMI, body mass index; **EEC**, endometrial echo-complex; **FSH**, follicle-stimulating hormone; **LH**, luteinizing hormone; **OSA**, obstructive sleep apnea; **QL**, quality of life; **REM**, rapid-eye-movement; **SD**, sleep disorders; **SDB**, sleep-disordered breathing.

Introduction

Earlier, we studied various metabolic aspects of sleep disturbances in the male and female population.[1,2] It is known that menopausal women experience various sleep-related disorders.[3-6] Menopause is characterized by a decrease in the production of female sex hormones, most specifically estrogen. According to the US National Library of Medicine,[7] about 16% to 42% of premenopausal women and 35% to 60% of postmenopausal women have sleep disorders (SD). There are many scientific data on sleep-related symptoms in menopausal women.[8-10] However, in the small number of studies of sleep-related symptoms there are reports of certain ethnic differences in sleep-related those symptoms experienced in the menopausal transition. These studies found no difference in sleep symptoms between black and white participants, but in a cross-sectional analysis among an elderly community cohort, performed by Mezick et al.,[11] snoring, sleepiness and long sleep duration were more common in Hispanics. The SWAN study (2008), which included Caucasian, African and Chinese menopausal women, described ethnic differences for staying asleep and

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early morning awakening. Further studies have evaluated the polysomnographic monitoring indicators, which also have revealed differences in the sleep pattern, showing that African women experience greater sleep latency, shorter total sleep time and lower sleep efficiency than do other ethnic groups. However, there are no studies of SD in Caucasian and Asian peri- and postmenopausal women in Russia. Some researchers have shown that Asian men (Chinese) had the highest rates of SDB among the 3 major ethnic groups.[12]

There are many nationalities living in East Siberia near Lake Baikal. The largest part of Asian representatives are Buryats, and of Caucasian representatives are Russians. The aim of our study was to define the structure of sleep in Caucasian and Asian peri- and postmenopausal women with sleep apnea.

Materials and Methods

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

Two hundred and forty-seven menopausal women between 45 and 60 years of age participated in this study between February 2016 and February 2018. The participants were divided into 2 ethnic groups, taking into account the genealogy (the representatives having in two generations of parents of one ethnic group) and self-identification, taking into account phenotype elements. Caucasians were represented by the Russian ethnic group (n=115) and Asians by the Buryat ethnic group (n=132).

Inclusion criteria were amenorrhea ≥12 months; a basal level of FSH>20iU/ml, LH/FSH ratio<1; ultrasounds criteria: (1) thin non-functional endometrium, the EEC measured <5mm; (2) the loss of ovarian reserve.

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

Most of the Caucasian women were recruited for study in Irkutsk and Asian women in Ulan-Ude (Byryat Republic). The study included the collection of anamnestic data, physical examinations, clinical (questionnaire, general medical examination, gynecological examination, polysomnographic monitoring), and biochemical laboratory methods. All participants were asked to take part in the follow-up questionnaire.

The questionnaire was available in Russian because it is the major language spoken in East Siberia. The questionnaire included self-reported sleep symptoms and the menopausal status. Menopausal status was assessed by self-reports based on the Modified Menopausal Index (MMI).

The Berlin questionnaire for OSA is one of the most commonly used. It combines risk factors, such as snoring, sleepiness, obesity and hypertension, to reliably predict OSA syndrome. According to this questionnaire, there are 2 levels of severity of the risk: a high risk (if there are 2 or more categories where the score is positive) and a low risk (if there is only 1 or no categories where the score is positive).[13 -16]

The polysomnographic monitoring was carried out in a specially equipped room, which was as close as possible to the conditions of a bedroom, using the GRASS-TELEFACTOR Twin PSG(Comet) system with an As 40 amplifier with the SPM-1(USA) integrated sleep module, according to the standard methodology. We evaluated overnight 16-channel polysomnography with 2 electroencephalograms (C4,C3,O1,O2), 2 electrooculograms (ROC, LOC) and 2 electromyogram channels; oral/nasal airflow by thermistor; respiratory effort via conductance belts on chest and abdomen; snoring sounds via microphone; oxygen saturation via pulse oximeter. Each 30-sec epoch was manually scored using standard scoring criteria supplemented by apnea-hypopnea criteria, according to American Academy of Sleep Medicine recommendations.[17]

Statistical analysis was performed using STATISTICA 6.1 software (Stat-Soft Inc., USA). Baseline characteristics were summarized as frequencies and percentages for categorical variables and as mean±standard deviation for continuous variables. Differences of continuous variables departing from the normal distribution were tested by the Mann-Whitney U-test. The frequencies of categorical variables were compared using Pearson $\chi 2$ or Fisher's exact test, when appropriate. A probability value of $P \leq 0.05$ was considered statistically significant.

Results

Results of sleep complaints and comparative analysis of sleep characteristics of Caucasian and Asian menopausal women are presented in Table 1. Sleep complaints, such as difficulty falling asleep, frequent night awakenings (more than 2 times), difficulties of morning awakening or problems waking up too early, were increased in perimenopausal Caucasian women. We found ethnic differences in a greater frequency of complaints in Caucasian women, but Asian women also showed a tendency to increased complaints about poor sleep. However, results of the Berlin questionnaire showed a high risk of having OSA syndrome in Asian postmenopausal women.

Basic physical characteristics of participants are summarized in Table 2. After the clinical-anamnestic method, the following stage of the research was polysomnographic monitoring in a sleep laboratory in participants with high and low risk of OSA syndrome. Results of polysomnographic characteristics in participants are presented in Table 3. OSA was more common in Asian postmenopausal women than in Caucasians. However, in structure of sleep we have not seen distinctions.

Discussion

Our study showed ethnic features of SD in menopausal women of 2 races living in East Siberia. Asian women self-reported frequent night awakenings and high risk of SDB, but more severe menopausal symptoms were found in White women compared to Asian women. It is known that a low level of female sex hormones can lead to SDB, which worsens QL.[18-20] The differences in the course of menopause in women of different races has been demonstrated in many studies. [21-25]

Table 1.

Result of self-reported sleep symptoms in Caucasian and Asian menopausal women

Characteristics	Caucasian women (n = 115)		Asian women (=132)		*P-value*
	Perimenopausal women (n=56) (1)	Postmenopausal women (n=59) (2)	Perimenopausal women (n=56) (3)	Postmenopausal women (n=76) (4)	
Difficulty falling asleep (more than 30 minutes), n (%)	39 (69.6)	32 (54.2)	29 (51.8)	46 (60.5)	P_{1-3}=0.05
Frequent night awakenings (more than 2 times), n (%)	15 (26.8)	37 (62.7)	27 (48.2)	40 (52.6)	P_{1-2}=0.000 P_{1-3}=0.019
Difficulties of morning awakening or problems waking up too early, n (%)	48 (85.7)	29 (49.2)	39 (69.6)	45 (59.2)	P_{1-3}=0.041
ESS (Epworth Sleep Scale)					
0-10, n (%)	17 (30.4)	21 (35.6)	20 (35.7)	46 (60.5)	P_{2-4}=0.004. P_{3-4}<0.005
10-24, n (%)	39 (69.6)	38 (64.4)	36 (64.3)	30 (39.5)	P_{2-4}<0.01 P_{3-4}<0.005
Berlin questionnaire					
Low risk n (%)	27 (48.2)	30 (50.8)	23 (41.1)	26 (34.2)	P_{2-4}=0.05
High risk, n (%)	29 (51.8)	29 (49.2)	33 (58.9)	50 (65.8)	P_{2-4}=0.05

Table 2.

Basic physical characteristics of Caucasian and Asian menopausal women

Characteristics	Caucasian women (n=115)		Asian women (n=132)		*P-value*
	SD+ n=70 (1)	SD- n=45 (2)	SD+ n=88 (3)	SD- n=44 (4)	
Age, yrs	54.34±4.81	53.25±5.18	54.92±5.04	54.1±4.32	
BMI, kg/m^2	30.14±2.21	27.39±4.27	31.02±2.89	28.91±1.26	P_{1-2}=0.000 P_{3-4}=0.000
Place of residence, n (%) Irkutsk, n (%) Ulan-Ude, n (%) Other region	 57 (81.4) 9 (12.8) 4 (5.7)	 39 (86.7) 6 (13.3) -	 11 (12.5) 77 (87.5) -	 6 (13.6) 38 (86.3) -	
Marital status, n (%) Married/partnered Nonmarried /separated	 48 (68.5) 22 (31.4)	 28 (62.2) 17 (37.8)	 67 (76.1) 21 (23.9)	 26 (59.1) 18 (40.9)	P_{3-4}=0.043
Menopausal status Perimenopause, n (%) Postmenopause, n (%)	 37 (52.8) 33 (47.14)	 19 (42.2) 26 (57.8)	 33 (37.5) 55 (62.5)	 23 (52.3) 21 (47.7)	P_{1-3}=0.05
MMI					
12-34, n (%) 35-58, n (%) >58, n (%)	20 (28.6) 28 (40.0) 22 (31.4)	14 (31.1%) 15 (33.3%) 16 (35.6%)	29 (33.0) 36 (40.9) 23 (26.1)	13 (29.5) 19 (43.2) 12 (27.3)	

Table 3.

Polysomnographic measures in Caucasian and Asian menopausal women with high and low risk of sleep apnea

Variable	Caucasian women (n=115)						Asian women (n=132)						*P-value*
	Perimenopausal (n=56)			Postmenopausal (n=59)			Perimenopausal (n=56)			Postmenopausal (n=76)			
	High risk n=6	Low risk n=38	SD- n=12	High risk n=11	Low risk n=37	SD- n=11	High risk n=18	Low risk n=21	SD- n=17	High risk n=22	Low risk n=37	SD- n=17	P<0.05
	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	
SE[1], %	71.74±8.4	95.2±3.5	96.2±2.2	87.3±6.3	90.5±2.7	92.5±5.9	75.2±5.3	93.7±5.1	96.3±5.1	74.5±8.5	90.3±4.73	94.3±3.7	P_{1-2} P_{1-3} P_{4-5} P_{4-6} P_{7-8} P_{7-9} P_{10-11} P_{10-12}
SL[2], min	28.1±6.4	16.2±3.7	19.2±6.1	26.3±3.3	19.9±6.5	22.3±18.2	25.2±6.1	19.5±2.7	21.2±8.5	19.1±5.8	20.2±5.6	21.8±5.4	
Stage (%)													
1	5.2±3.5	6.8±3.4	5.5±1.6	6.5±1.6	10.2±3.5	7.6±4.1	6.0±4.8	8.2±4.1	7.2±4.1	7.4±3.2	6.5±2.9	6.4±2.4	
2	65.0±18.9	57.7±13.4	58.2±15.9	60.2±19.2	54.1±15.6	53.9±7.0	60.2±20.5	61.5±18.2	56.9±24.3	69.8±24.1	55.1±16.2	53.2±20.1	
3	12.1±4.9	19.1±6.4	23.2±5.8	12.2±5.4	18.5±6.4	19.4±3.8	11.2±5.8	18.1±5.4	20.5±6.1	10.1±5.2	16.2±5.8	18.5±61	
REM	19.8±5.1	21.1±5.4	20.1±5.6	20.6±8.2	21.6±9.3	25.8±4.3	16.1±5.9	15.2±6.4	16.8±5.4	14.5±5.1	22.8±5.3	21.8±6.4	P_{4-10} P_{10-11} P_{10-12}
AGI[3], event/ hour	13.2±2.4	7.1±1.2	6.2±0.5	15.2±3.4	8.5±0.5	7.1±0.1	18.2±3.8	8.1±2.1	6.4±4.1	22.1±2.5	9.2±4.1	6.2±1.2	P_{1-2} P_{1-3} P_{1-7} P_{4-5} P_{4-6} P_{4-10}
O_2 saturation, (%)	90.1±3.1	95.2±4.2	96.4±3.5	90.1±4.5	93.3±0.5	96.2±2.1	91.1±3.8	92.4±2.6	92.1±2.1	88.1±4.1	89.1±3.1	91.1±4.4	P_{4-10}

[1]. *SE (leep efficiency) is a percentage computed as the total time spent asleep / the total amount of time spent in bed × 100*

[2]. *SL (sleep latency) was calculated as time from beginning of the recording period to the first of 10 consecutive min of stage 2 or stage 3-4 sleep*

[3]. *AGI - apnea-hypopnea index*

Results of our study showed differences in SD in Caucasian and Asian women. Although, the SWAN study involving White, African American and Chinese women has demonstrated that African women have the most severe night vasomotor reactions compared to Asian and White women. Moreover, the best sleep patterns have been shown in Asian women compared to Whites and African Americans,[26] and our results confirmed this finding when comparing Asian and Caucasian women. Thus, Caucasian women, compared to Asians, have a high frequency of difficulty falling asleep and difficulties of morning awakening. However, Asian women have a high risk of SDB, both in the survey data and according to polysomnography. In this regard, we should emphasize a certain type of Asian physique with a short neck and trunk that was formed due to the centuries-old residence in a sharply continental climate with a hot summer to 38°C and frosty winters to -50°C.

There were no differences in sleep architecture between ethnic groups. It has been found that the representation of REM is greater in perimenopausal women than in postmenopausal ones, which can be explained by a change in sleep structure with age—decreasing delta-sleep and some compensatory increasing REM. However, some studies have demonstrated that there is a decreasing REM duration in normal aging.[7] According to our results, it is possible that worsening of the vital status contributes to SD, which is also reported in some other studies.

Results of our pilot study are not complete and need to be continued. However, for improving QL, it is necessary not only to pay attention to menopausal problems, but also to solve the sleep problems associated with various strategies, both age management and lifestyle, which have been stressed in a study by Woods et al.[27] We believe that management strategies should be oriented towards ethnic features related to lifestyle and the type of response to aging.

Conflict of interest

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

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Oxidative Stress Parameters and State of Regional Periodontal Blood Flow in Adolescents with Arterial Hypertension and Periodontal Diseases

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Abstract

The aim of this research was to investigate LPO activity in adolescents with arterial hypertension (AH) and periodontal disease (PD) and to establish the relationship between LPO-AOD parameters and the state of regional periodontal blood flow.

Materials and Methods: A total of 113 adolescent boys and girls participated in the research (age range of 12 to 17 years). The participants were divided into 3 groups: a comparison group with PD, a clinical group with AH and without PD, and a clinical group with AH and PD. We estimated the plasma level of antioxidant parameters (total antioxidant activity, SOD activity, α-tocopherol and retinol) and primary/secondary products of LPO (conjugated dienes, ketodienes and conjugated trienes, and thiobarbituric acid reactive substances [TBARS]) using spectrophotometric and fluorometric methods. Doppler ultrasound was used to assess the hemodynamics of the microvasculature of the periodontium: the resistivity index (RI) and pulsation index (PI) were calculated.

Results: our results indicate an increase of the imbalance in LPO-AOD system in patients with AH and PD. This imbalance is manifested in the accumulation of TBARS, thus decreasing the activity of antioxidant protection factors (α-tocopherol and SOD), as well as in the presence of pathological relationships between the parameters of LPO and indicators characterizing a decrease in vascular blood flow. These changes indicate the rapid involvement of LPO processes in the pathogenic mechanisms of developing structural and functional disorders of periodontal tissues in AH. These changes may be associated with a pronounced functional overload of periodontal tissues in the presence of inflammation. The changes in the velocity characteristics of blood flow in periodontal tissues in adolescents with AH are associated with increasing the toxic lipoperoxide metabolites and are important diagnostic criteria of regional hemodynamic disorders.(**International Journal of Biomedicine. 2018;8(4):301-305.**)

Key Words: arterial hypertension • adolescents • lipid peroxidation • antioxidant defense • periodontal diseases

Abbreviations

AH, arterial hypertension; **AOD**, antioxidative defense; **CDs**, conjugated dienes; **KD-CT**, ketodienes and conjugated trienes; **LPO**, lipid peroxidation; **PD**, periodontal disease; **PI**, pulsation index; **RI**, resistivity index; **SOD**, superoxide dismutase; **TAA**, total antioxidant activity; **TBARS**, thiobarbituric acid reactive substances; **Vas**, blood flow velocity in the arterial systole.

Introduction

Arterial hypertension (AH), common in adults, for a long time has been considered a rare childhood pathology. However, many studies have found elevated blood pressure in childhood.[1-3] Despite the fact that the mechanisms of AH formation have been studied in detail, the metabolic changes leading to its early development in adolescents are not yet clear. It has been found that systemic hemodynamic disorders are characterized by metabolic and functional disorders in all organs and systems

of the body, including tissues of the dentoalveolar system.[4,5] Thus, it is noted that the microcirculation of the periodontium due to abundant vascularization of tissues and more subtle organization of vessels is extremely sensitive to the slightest vascular fluctuations.[3] In recent years, important results have been obtained on the influence of hypertension on the vessels of periodontal tissues. Unfortunately, the studies dedicated to the state of the dental system in children and adolescents with AH are rare and do not give sufficient information about this issue. It is considered to be a proven fact that an increase in the production of free radicals or oxidative stress plays a key role in the development of a number of cardiovascular diseases, including hypertension.[6] It has been found that oxidative stress acts as a leading pathogenetic factor of endothelial dysfunction in primary (essential) hypertension, and its severity positively correlates with the severity of hypertension.[7] At the same time, the disorganization of homeostatic mechanisms of the periodontal microcirculation in hypertension has been identified as a cause of chronic tissue hypoxia of the periodontal complex, in which the processes of LPO of biomolecules are activated, leading to a violation of the structure and function of the periodontal biomembranes. [8] However, there are still not enough investigations to clarify the relationship between changes in regional blood flow and LPO processes in children and adolescents with hypertension and the impact of these changes on the manifestations of major dental diseases in childhood.

The aim of this research was to investigate LPO activity in adolescents with AH and PD and to establish the relationship between LPO-AOD parameters and the state of regional periodontal blood flow.

Material and Methods

A total of 113 adolescent boys and girls participated in the research (age range of 12 to 17 years). The participants were divided into 3 groups: a comparison group (Group1) with PD (n=37, mean age of 14.57±2.01 years), a clinical group (n=19, mean age of 15±1.53 years) with AH and without PD (Group 2), and a clinical group (n=57, mean age of 14.7±1.89 years) with AH and PD (Group 3).

The common inclusion criterion for all groups was either voluntary informed consent of teenagers 15 years of age or older or of the parents/legal representatives of the adolescents. Exclusion criterion for all groups was intake of anti-oxidant drugs within the last 6 months.

The main inclusion criterion for Groups 2 and 3 was confirmed AH based on the measurement of blood pressure in repeated office measurements ≥95 percentile for age, height and sex or ≥140/90 mmHg in adolescents older than 16 years. Exclusion criteria for Groups 2 and 3 were secondary hypertension and the presence of severe somatic diseases. Additional inclusion criteria for Group 3 were the presence of inflammatory PD, the absence of malocclusion, abnormal position of teeth, and the lack of current orthodontic treatment. Inclusion criteria for Group 1 were comparability in age, sex, place of residence and the additional inclusion criteria for Group 3.

Clinical dental examination of each teenager was carried out according to the generally accepted method and included a survey, external examination, and oral cavity examination. Complex periodontal index was determined during dental examination. A moderate PD was found in the most patients of Groups 1 and 3.

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.

Blood samples (5 ml) were collected from the ulnar vein in standard vacuum tubes with EDTA. 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 ethylenediaminetetraacetic acid plasma and washed erythrocytes were used immediately or kept frozen at -40°C, not exceeding one month. We estimated the intensity of LPO-AOD parameters by plasma concentrations of antioxidant parameters (TAA, SOD activity, α-tocopherol and retinol) and primary/secondary products of LPO (CDs, KD-CT, and TBARS). The concentration of CDs and KD-CT was detected at 232 nm in plasma heptane extracts.[9] For conversion of absorption units to μmol/L, we used the coefficient of molar absorption ($K=2.2\cdot10^5 M^{-1}C^{-1}$). TBARs levels, SOD activity in hemolysate and α-tocopherol and retinol levels in plasma were detected by fluorometry.[9] Blood plasma TAA level was detected photometrically.[9] The measurements were conducted with a Shimadzu RF-1501 spectrophotometer (Japan) consisting of two blocks: a UV-1650PC spectrophotometer and a RF-1501 spectrofluorimeter.

Doppler ultrasound was used to assess the hemodynamics of the microvasculature of the periodontium. The study of periodontal tissues was carried out with a Mindray DC-8. The state of blood flow in the periodontal vessels was determined by quantitative analysis of Doppler curves and was based on the assessment of the time-averaged maximum value of the blood flow velocity in the systole (Vas, units). The following parameters were calculated: RI (units), reflecting the resistance to blood flow distal to the measurement site and PI (units), reflecting the elastic properties of the arteries and decreases in those properties with age.

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), median (Me), interquartile range (IQR; 25th to 75th percentiles). 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. 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 Group 3, the levels of CDs and α-tocopherol were significantly lower than in Group 1, but the levels of TBARS and retinol were significantly greater. Intergroup differences between groups with AH showed that the levels of KD-CT and SOD activity were significantly lower in Group 3 than in Group 2 (Table 1).

Table 1.

The level of LPO products and AOD parameters in the studied groups (M±SD, Me, IQR [P_{25}; P_{75}])

Parameters	Group 1 (1)	Group 2 (2)	Group 3 (3)	Statistical significance of $P<0.05$
CDs, µmol/L	1.54±0.68 1.67 0.81 - 2.12	1.33±0.81 1.14 0.56 - 2.02	0.99±0.63 0.98 0.51 - 1.48	P_1-P_3
KD-CT, units	0.33±0.14 0.28 0.22 - 0.42	0.49±0.29 0.36 0.32 - 0.52	0.38±0.12 0.36 0.27 - 0.48	P_2-P_3
TBARS. µmol/L	0.78±0.3 0.76 0.62 - 0.9	0.81±0.32 0.83 0.61 - 0.87	1.09±0.52 0.98 0.65 - 1.48	P_1-P_3
TAA. units	13.86±3.78 13.87 12.12 - 16.31	15.84±4.55 16.17 13.22 - 19.86	14.76±6.69 13.8 10.15 - 17.62	
SOD activity, units	1.58±0.17 1.58 1.44 - 1.72	1.64±0.19 1.67 1.56 - 1.73	1.54±0.16 1.55 1.41 - 1.64	P_2-P_3
α-tocopherol, µmol/L	7.05±2.95 7.32 4.9 - 8.43	6.23±3.73 4.99 4.12 - 5.84	5.41±1.82 5.1 4.03 - 6.27	P_1-P_3
Retinol, µmol/L	0.72±0.34 0.65 0.48 - 0.93	0.92±0.63 0.78 0.62 - 0.93	0.9±0.35 0.87 0.66 - 1.15	P_1-P_3

In addition, Group 3 was characterized by increased values of Vas and RI compared to Group 1. Intergroup differences between groups with AH showed that the levels of Vas and PI were significantly lower in Group 3 than in Group 2 (Table 2).

Table 2.

Parameters of periodontal microvasculature in adolescents of the studied groups (M±SD, Me, IQR [P_{25}; P_{75}])

Parameters	Group 1	Group 2	Group 3	Statistical significance of $P<0.05$
Vas, units	30.85±6.06 32.13 29.01 - 36.18	45.93±4.34 47.2 41.4 - 50.53	34.66±6.11 35.13 32.19 - 41.4	P_1-P_3 P_2-P_3
PI, units	2.17±0.67 2.14 1.8 - 2.57	2.31±0.67 1.97 1.89 - 2.72	2.01±0.29 2.09 1.98 - 2.21	P_2-P_3
RI, units	0.68±0.07 0.69 0.64 - 0.72	0.84±0.05 0.83 0.81 - 0.86	0.81±0.04 0.81 0.79 - 0.83	P_1-P_3

In Group 1, correlation analysis found the following significant correlations: Vas–PI (r=-0.68), PI–RI (r=0.46), RI–α-tocopherol (r=-0.45), and RI–CDs (r=-0.45). In Group 2, the following significant correlations were found: Vas–RI (r=-0.56), PI–RI (r=0.81), KD-CT–retinol (r=0.63), PI–TAA (r=0.63), and RI–TAA (r=0.7). Group 3 was characterized by a significant number of intra-system correlations (Vas–PI [r=0.46], Vas–RI [r=-0.31], and CDs–TAA [r=-0.34]) and inter-system correlations (Vas –TBARS [r=-0.3] and RI – TBARS [r=0.33]).

Data analysis revealed a slight decrease in the level of LPO primary products with a significant accumulation of TBARS in the adolescents of Group 3. This can indicate a rapid transition of products from primary to end metabolites. It is known that most LPO products in excess concentrations are highly toxic and have a damaging effect on the structural components of cells.[10-12] For example, the lipid hydroperoxides are able to inhibit DNA synthesis, induce apoptosis and, thereby, suppress proliferation, maturation and growth of cells; also, they have teratogenic and carcinogenic effects, which can cause mutations.[13] As a rule, an increase of the LPO end products indicates the rapid involvement of LPO processes in the pathogenetic mechanisms that develop the structural and functional disorders in the cells of organs and tissues. In Group 3, these changes occurred due a sharp decrease in the level of α-tocopherol and an increase in the level of retinol. It is considered that α-tocopherol is oxidized while performing its antioxidant function; its recovery occurs with the help of other antioxidants, such as retinol and ascorbate.[14,15] In such a case, we can assume a compensatory increase in the retinol level in conditions of α-tocopherol deficiency. The reduced activity of SOD revealed in the development of periodontal inflammation and AH can have a negative impact on the state of the biomembranes of periodontal tissue cells. In pathological conditions, the active forms of oxygen are able to inhibit the activity of the antioxidant enzymes. For example, hydroperoxides inhibit the activity of SOD,[16,17] damaging its structure with LPO toxic products.[18,19] An important factor of the AH pathogenesis is the microcirculation disorder that can serve as the basis of inflammatory and destructive periodontal diseases.[6] This disorder can be manifested through various changes in the permeability of microvessels, aggregation of platelets and erythrocytes, flexibility of red blood cells, volumetric tissue blood flow and oxygen delivery to tissues.[20-22] In fact, the pathogenetic manifestations of AH are carried out by disorders of microcirculation and the oxygen balance of tissues. The severity, course and outcome of the cardiovascular diseases, including AH, are largely determined by these kinds of disorders. Our study of the state of blood flow in periodontal tissues showed that the changes in the velocity characteristics depend on the presence or absence of systemic disorders. From our point of view, the reduction of the maximum blood flow rate in the systole is associated with spasm of arterioles, stagnation in the venous microcirculatory vessels and severe rheological disorders in the periodontium in patients with AH.[23] We found that a decrease in BP damping function occurs with a decrease in the arterial wall extensibility (i.e., with an increase in its stiffness).[24,25]

Therefore, it is of great importance to identify possible factors affecting the development of anatomical and functional changes in the microcirculatory vessels.[4,26] Damaging factors may be strengthened by the accumulation of prooxidants in the periodontal tissues and their subsequent disintegration with the formation of free radicals, which have a destructive effect on the vascular wall, leading to fibrosis, thickening of capillaries, and partial or complete obliteration.[5,22] Despite the age of the participants, our data are close to the investigations of atherosclerosis, arteriosclerosis and calcification of the vascular wall, which are considered to be the reasons for the decrease in the elasticity of the large arterial vessels.[27,28]

Conclusion

Thus, our results indicate an increase of the imbalance in LPO-AOD system in patients with arterial hypertension and periodontal disease. This imbalance is manifested in the accumulation of thiobarbituric acid reactive substances (LPO end products), thus decreasing the activity of antioxidant protection factors (α-tocopherol and SOD), as well as in the presence of pathological relationships between the parameters of LPO and indicators characterizing a decrease in vascular blood flow. These changes indicate the rapid involvement of LPO processes in the pathogenic mechanisms of developing structural and functional disorders of periodontal tissues in arterial hypertension. These changes may be associated with a pronounced functional overload of periodontal tissues in the presence of inflammation. The changes in the velocity characteristics of blood flow in periodontal tissues in adolescents with arterial hypertension are associated with increasing the toxic lipoperoxide metabolites and are important diagnostic criteria of regional hemodynamic disorders.

Conflict of interest

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

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Characteristics of Lipid Peroxidation Processes and Antioxidant Status in Teenagers-Boys of Different Ethnic Groups with Exogenous Constitutional Obesity and Non-Alcoholic Fatty Liver Disease

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Abstract

The aim of this research was to study changes in the LPO-AOD processes in Mongoloid and Caucasian teenagers with exogenous constitutional obesity (ECO), including those complicated by non-alcoholic fatty liver disease (NAFLD).

Materials and Methods: A total of 18 Mongoloid teenage boys and 17 Caucasian teenage boys with NAFLD (Clinical group 1 and Clinical group 2, respectively) on the background of ECO of the first degree were examined. For comparison, data of 37 apparently healthy Mongoloid teenage boys (Control group 1) and 23 Caucasian teenage boys (Control group 2) was used. The plasma level 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 reactive substances [TBARS]) were determined using spectrophotometric and fluorometric methods.

Results: Evaluation of the activity of LPO reactions in Clinical group 1 indicated an increase in the content of compounds with DB relative to Control group 1. A similar trend was found in the concentration of CD and KD-CT. In Clinical group 2, we found a statistically significant increase only in the values of KD-CT. In the parameters of the AOD system, multidirectional changes of patients parameters compared with the control group were observed in Clinical group 1: the increased values of TAA, SOD activity, and reduced levels of α-tocopherol, retinol, and GSSG. In Clinical group 2, lower values of α-tocopherol, GSSG, and SOD activity were observed relative to Control group 2. Differences between the two ethnic groups were present in the control groups—the reduced level of TAA, GSH and the increased level of GSSG in the group of Mongoloids; just as in clinical groups with NAFLD, a high level of SOD activity was observed in Mongoloids compared to Caucasians.

Conclusion: The changes revealed in the redox state in Mongoloid and Caucasian teenagers with ECO and NAFLD indicate high activity of LPO processes and severe deficiency of antioxidant vitamins in patients of both ethnic groups. The obtained results allow us to recommend administration of antioxidant drugs in addition to courses of metabolic therapy in comprehensive treatment of patients with ECO and NAFLD. (**International Journal of Biomedicine. 2018;8(4):306-310.**)

Key Words: lipid peroxidation • antioxidant defense • teenage boys • obesity • non-alcoholic fatty liver disease

Abbreviations

AOD, antioxidative defense; **BW**, body weight; **BMI**, body mass index; **CD**, conjugated dienes; **DB**, unsaturated double bonds; **GSH**, reduced glutathione; **GSSG**, oxidized glutathione; **ECO**, exogenous constitutional obesity; **KD-CT**, ketodienes and conjugated trienes; **LPO**, lipid peroxidation; **NAFLD**, non-alcoholic fatty liver disease; **SOD**, superoxide dismutase; **TAA**, total antioxidant activity; **TBARS**, thiobarbituric acid reactive substances.

Introduction

Non-alcoholic fatty liver disease (NAFLD) is considered to be a hepatic manifestation of metabolic syndrome, and therefore its prevalence is directly associated with the growth in obesity among the population.[1,2] NAFLD (liver steatosis, fatty infiltration, and fatty liver) is a condition in which more than 5% of the liver mass consists of fat, mostly triglycerides. If in addition to excessive accumulation of fat, an active inflammatory process takes place, it indicates the progression of the disease—non-alcoholic steatohepatitis, which significantly increases the risk of developing more serious liver diseases.[3,4] Insulin resistance and hypertriglyceridemia are observed in patients with NAFLD; therefore, various methods of correcting overweight are often used, including lipotropic drugs and antioxidants.[5-8] The results of numerous clinical studies indicate an increase in the incidence of exogenous constitutional obesity in teenagers, especially among boys; hence, studying NAFLD, being a frequent concomitant disease in obesity, is highly relevant.[9-12] Currently, for this category of patients it seems appropriate to study changes in the LPO-AOD system, as well as to administer a range of antioxidants, individually selected according to the nature of the imbalance detected.[13-16] It is also necessary to consider a patient's ethnicity when making a diagnosis and conducting differentiated health-improving programs and therapy measures.[17,18]

The aim of this research was to study changes in the LPO-AOD processes in Mongoloid and Caucasian teenagers with ECO, including those complicated by NAFLD.

Materials and Methods

A total of 18 Mongoloid teenage boys (mean age of 13.81±2.23 years) and 17 Caucasian teenage boys (mean age of 14.87±1.59 years) with NAFLD (Clinical group 1 and Clinical group 2, respectively) on the background of ECO of the first degree were examined. For comparison, data of 37 apparently healthy Mongoloid teenage boys (mean age—15.14±0.88 years) (Control group 1) and 23 Caucasian teenage boys (mean age of 14.33±1.86 years) (Control group 2) was used. All patients underwent inpatient treatment in Children's Republican Clinical Hospital of the Ministry of Health of the Republic of Buryatia (Ulan-Ude).

Inclusion criteria for clinical groups: age range of 13 to 17 years, excess body weight of more than 95 percentile for a given height, age and sex; absence of acute, or exacerbation of chronic diseases at the time of inclusion in the study and at least one month before it.

Exclusion criteria: symptomatic and genetic forms of obesity; intake of medications that could affect body weight and estimated metabolic parameters. The criteria for the diagnosis of NAFLD: ECO, diffuse changes of liver according to ultrasound and computed tomography of the abdominal cavity, absence of cytolysis (normal levels of ALT and AST), and exclusion of infectious etiology of hepatitis.

Blood samples were collected in accordance with the existing requirements in the morning on an empty stomach from the cubital vein. All teenagers were subjected to general clinical examination, including anamnestic data collection, physical examination, anthropometric data analysis (BW, height, BMI), blood pressure measurement, nutritional status assessment and determination of concentration of total cholesterol and triglycerides in blood serum, glucose tolerance testing.

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.

The intensity of LPO processes was assessed by the content of DBs, primary/secondary products of LPO by the method of I.A. Volchegorskiĭ,[19] based on the intensive absorption of conjugated diene structures by lipid hydroperoxides in the range 220 nm, 232 nm, and 278 nm. The content of TBARS was determined in the reaction with thiobarbituric acid using the fluorimetric method of V.B. Gavrilov et al.[20] Total antioxidant activity (TAA) was evaluated by the method of G.I. Klebanov et al.[21]

To evaluate TAA, we used a model system, which is a lipoprotein suspension of chicken egg yolk that allows evaluating the ability of blood serum to inhibit the accumulation of TBARs in suspension. LPO was induced by adding $FeSO_4 \times 7H_2O$.

Concentrations of α-tocopherol and retinol were determined using the method of R.Ch. Chernyauskene.[22] The method provides for the removal of substances that prevent determination by saponification of samples in the presence of large amounts of ascorbic acid and extraction of unsaponifiable lipids with hexane, followed by fluorometric determination of the content of α-tocopherol and retinol. At this, α-tocopherol has intense fluorescence with maximum excitation at λ= 294 nm and radiation at λ=330 nm; retinol - at λ= 335 nm and λ= 460 nm. The content of GSH and GSSG was determined by P.Y. Hissin and R. Hilf,[23] the SOD activity was determined by H.P. Misra and I. Fridovich.[24] The measurements were conducted with a Shimadzu RF-1501 spectrophotometer (Japan) consisting of two blocks: a UV-1650PC spectrophotometer and a RF-1501 spectrofluorimeter.

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 median (Me) and interquartile range (IQR; 25th to 75th percentiles). Differences between groups were tested by the Mann-Whitney U-test. A probability value of $P<0.05$ was considered statistically significant.

Results and Discussion

Analysis of anthropometric data in both clinical groups showed a statistically significant increase in BW ($P<0.001$) and BMI ($P<0.001$) compared to the control groups. At the same time, there were no statistically significant differences between clinical groups (Table 1). Thus, the studied clinical groups were comparable in their main anthropometric indices.

Table 1.

Anthropometric data of teenage boys of different ethnic groups (Me, IQR [P_{25}; P_{75}])

Parameters	Mongoloids		Caucasians	
	Control group 1 (n=37)	Clinical group 1 (n=18)	Control group 2 (n=23)	Clinical group 2 (n=17)
BW, kg	45.5 (44.0; 50.0)	71.0 (63.6; 72.5)*	63.5 (50; 68)	88.4 (74.3; 113.1)*
Height, cm	154 (150.3; 157.5)	155 (150; 163)	170 (160;176)	169 (163; 184)
BMI, kg/m^2	19.4 (18.5; 20.5)	28.0 (28.0; 28.7)*	21.9 (19.4; 22)	30.8 (29.5; 37.3)*

**- P<0.05 - compared to the control group.*

Evaluation of the activity of LPO reactions in Clinical group 1 indicated an increase in the content of compounds with DB (P=0.0013) relative to Control group 1 (Table 2). A similar trend was found in the concentration of CD (P=0.0017) and KD-CT (P<0.0001). An increase in the values of KD-CT turned out to be significant—more than 4 times. In Clinical group 2, we found a statistically significant increase only in the values of KD-CT (P=0.0025) (Table 2). The level of TBARS was not statistically changed in both clinical groups compared to control groups (P>0.05). Interethnic differences were related only to control groups and resulted in lower values of compounds with DB (P<0.0001) and CD (P<0.0001), and increased levels of TBARS in Mongoloid boys, compared to Caucasians (Table 2).

Table 2.

State of LPO system in teenage boys of different ethnic groups (Me, IQR [P_{25}; P_{75}])

Parameters	Mongoloids		Caucasians	
	Control group 1 (n=37)	Clinical group 1 (n=18)	Control group 2 (n=23)	Clinical group 2 (n=17)
Compounds with conjugated DB, units	1.02 (0.84; 1.5)	1.83 (1.49; 2.07)*	2.58 (1.38; 3.7)^	2.03 (1.86; 2.34)
CD, μmol/L	0.78 (0.52; 0.96)	1.22 (1.04; 1.42)*	2.42 (1.96; 2.94)^	1.6 (1.52; 1.82)
KD-CT, units	0.14 (0.1; 0.2)	0.67 (0.42; 0.85)*	0.26 (0.14; 0.38)	0.48 (0.32; 0.72)*
TBARS, μmol/L	1.15 (1.08; 0.8)	0.95 (0.77; 1.22)	0.71 (0.61; 0.96)^	1.26 (0.87;1.41)

**-P<0.05 - compared to the control group*
^- P<0.05 - between two control groups

In the parameters of the AOD system, multidirectional changes of patients parameters compared with the control group were observed in Clinical group 1: the increased values of TAA (P=0.0024), SOD activity (P=0.0004), and reduced levels of α-tocopherol (P<0.0001), retinol (P<0.0001), and GSSG (P=0.0083) (Table 3). In Clinical group 2, lower values of α-tocopherol (P=0.0012), GSSG (P=0.018), and SOD activity (P=0.0099) were observed relative to Control group 2. Differences between the two ethnic groups were present in the control groups—the reduced level of TAA (P<0.0001), GSH (P=0.0017) and the increased level of GSSG (P=0.0009) in the group of Mongoloids; just as in clinical groups with NAFLD, a high level of SOD activity (P=0.0011) was observed in Mongoloids compared to Caucasians (Table 3).

Table 3.

State of AOD system in teenage boys of different ethnic groups (Me, IQR [P_{25}; P_{75}])

Parameters	Mongoloids		Caucasians	
	Control group 1 (n=38)	Clinical group 1 (n=18)	Control group 2 (n=23)	Clinical group 2 (n=17)
TAA, units	7.27 (6.14; 9.24)	13.51 (6.02; 16.42)*	15.48 (13.62; 19.01)^	16.88 (15.4; 19.17)
α-tocopherol, μmol/L	6.48 (5.04; 7.96)	3.6 (2.78; 4.69)*	7.08 (5.95; 9.95)	3.66 (3.45; 4.72)*
retinol, μmol/L	0.61 (0.56; 0.68)	0.43 (0.34; 0.46)*	0.59 (0.54; 0.75)	0.41 (0.33; 0.52)
SOD activity, units	1.66 (1.6; 1.72)	1.85 (1.83; 1.87)*	1.74 (1.64; 1.89)	1.45 (1.37; 1.8)*#
GSH, mmol/L	1.89 (1.8; 2.07)	1.93 (1.76; 2.51)	2.22 (2.04; 2.46)^	2.18 (1.76; 2.33)
GSSG, mmol/L	2.24 (2.05; 2.39)	1.94 (1.67; 2.22)*	2.00 (1.84; 2.14)^	1.72 (1.41; 1.79)*

**-P<0.05 - compared to the control group*
^- P<0.05 - between two control groups
#- P<0.05 - between two clinical groups

In our study, an intensive accumulation of the secondary LPO products was recorded in both Mongoloid and Caucasian teenagers with obesity and NAFLD. Various studies have shown that ECO is characterized by increased intensity of free radical reactions that contribute to the accumulation of toxic LPO products in the blood.[25,26] Serious metabolic liver disorders in obesity, when excessive accumulation of lipids in hepatocytes occurs, can contribute to the mentioned disorders in the redox state in the presence of NAFLD.[27] Regular intensification of free radical oxidation of hepatic lipids leads to an increase in the content of free fatty acids, a decrease in the rate of their oxidation in mitochondria, an increase in the level of triglycerides, cholesterol in the blood, etc.[3,28] Hypersecretion of proinflammatory cytokines was also observed, including tumor necrosis factor-α, interleukin-6, and interleukin-8.[5] The result of these pathological reactions is hepatocyte necrosis, development of inflammatory cell infiltration, and subsequent activation of fibrogenesis.[3,5,6] Thus, excessive accumulation of toxic LPO products may exacerbate existing damage, preceding the appearance of more serious changes in the metabolism.

It is known that the parameters of the AOD system are a significant diagnostic criterion of adaptation reactions of the body.[14,15] The increase in TAA values as an integral parameter of the AOD system in Mongoloid teenagers with obesity and NAFLD may indicate activation of compensatory reactions in this group of patients, which is also confirmed by the increased activity of the main antioxidant enzyme–SOD. At the same time, there is a severe deficiency of antioxidant vitamins, α-tocopherol and retinol in these patients. In the group of Caucasians with NAFLD, there is also a drastically reduced level of some components of AOD system—α-tocopherol, GSSG, and SOD activity. It has been established that α-tocopherol and retinol are natural antioxidants and necessary nutritional factors.[15,22,29] Thus, α-tocopherol has a high membrane protective and antimutagenic activity, whereby interacting with natural antioxidants of other classes, it acts as an essential regulator of oxidative homeostasis of cells and tissues.[29] The antioxidant function of retinol is expressed in the protection of biomembranes from damage by active forms of oxygen.[30] It is known that the liver is the main pool of fat- and water-soluble vitamins; therefore, we confirmed in our study the decrease in the content of these vitamins in liver damage and their corresponding deficiency at the system level in the teenagers of both ethnic groups. However, interethnic data analysis showed an increased activity of the main antioxidant enzyme SOD in Mongoloid teenagers compared to Caucasians, which may indicate the activation of compensatory processes in the Mongoloid group.

Conclusion

The changes revealed in the redox state in Mongoloid and Caucasian teenagers with ECO and NAFLD indicate high activity of LPO processes and severe deficiency of antioxidant vitamins in patients of both ethnic groups. Interethnic differences in observed groups were characterized by increased activity of SOD in Mongoloids in comparison with Caucasians. The obtained results allow us to recommend administration of antioxidant drugs in addition to courses of metabolic therapy in comprehensive treatment of patients with NAFLD.

Conflict of interest

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Environmental Health

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Space Weather and Its Impact on Psycho-Emotional State of the Inhabitants of Different Latitudes

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Abstract

Purpose of the research was to conduct a comparative analysis of the psychological characteristics of the inhabitants of the northern and middle latitudes, depending on their psychological sensitivity to changes in geomagnetic disturbances

Materials and Methods: The study included 78 relatively healthy volunteers (women and men, the average age of 44.2[36.7; 54.3] years) living in the polar (Tiksi), subpolar (Yakutsk) and middle (Saratov) latitudes. Monitoring of the studied indicators was carried out during March and April 2016. To achieve this goal, the following methods were used: the Spielberger-Khanin scale for reactive (situational) and personal anxiety; E. Heim's technique for revealing individual coping strategies; the projective psycho-geometric test. Every day, during March and April 2016, the Kp-index, an integral indicator of changing geomagnetic disturbance was used. Depending on the identified matches, the peak values of the reactive anxiety by Spielberger-Khanin and Kp-index (not less than 60% of cases), all volunteers were divided into 2 groups. Group 1 included persons with similar overlap and, therefore, carriers of psychological sensitivity to changes in the action of heliogeomagnetic factors. Group 2 included persons without a specified sensitivity (i.e., no coincidences).

Results: In the course of the study, both coinciding and latitude-related differences in the psychological characteristics of different groups of volunteers were established. In the inhabitants of the polar and subpolar latitudes, especially in Group 1, the behavioral sphere was the most vulnerable (no constructive coping strategies). In addition, their changes in the emotional sphere contributed to the manifestation of aggressiveness and suppression of emotions against the background of general concentration, purposefulness and integrity of the individual. Regardless of the latitude of residence, volunteers, psychologically sensitive to changes in heliogeomagnetic factors, were more constructive in the emotional sphere, and the least in the behavioral, which gives reason to state that, depending on the factors of space weather, the behavior of the individual is the most vulnerable.(**International Journal of Biomedicine. 2018;8(4):311-316.**)

Key Words: heliogeomagnetic factors • anxiety • coping behavior • psychological sensitivity • multi-latitude monitoring

Introduction

"We all live in the crown of the Sun" is the indisputable thesis of A.L. Chizhevsky. But how is human health related to changes in solar activity and the geomagnetic field? The full answer to this question has not yet been found.

From 2003 to 2010, through the joint efforts of physicists, physicians, psychologists, information technology specialists, and biologists, a unique, large-scale international telecommunication project "Heliomed" was carried out, where the impact of space weather on the human body was studied by a single online protocol, on identical equipment, in medical centers located at different latitudes.[(1)]

Since 2014, the village of Tiksi (polar latitude) and the cities of Yakutsk (subpolar latitude) and Saratov (middle latitude) have been undergoing multi-latitude monitoring aimed at studying the influence of space weather factors on the nervous and cardiovascular systems of relatively healthy

volunteers, which was a continuation of the "Heliomed" project.[2] Stages of monitoring are held in the periods of maximal activity of the heliogeophysical factors – fall and/or spring.

Tiksi and Yakutsk are located in the territory of the Republic of Sakha (Yakutia). It is interesting that in this region in a wide range of longitude there is the greatest difference in the slopes of the Earth's axis of rotation and the axis of its geomagnetic field, which is about 11^0. On our planet, there is only one region with similar properties, but because it is located in the Atlantic Ocean, research cannot be conducted there, and, consequently, the results obtained in the course of this monitoring acquire special scientific value.

Recently, a major role in the development of socially significant pathology, including cardiovascular disease, which is the «culprit» of high morbidity and mortality in the adult population of the planet, is played by stress and emotionally negative risk factors (such as depression, increased level of neurotization, anxiety and a number of others).[3-7] In addition, it has been shown that the same elevated levels of neurotization, depression and anxiety in clinically healthy individuals can be a significant risk factor for diseases in the pathogenesis of which vasomotor dysfunction of the endothelium is the trigger.[8]

Data on the assessment of the psycho-emotional status of persons under the influence of space weather factors in the domestic and foreign literature are not widely presented. In fact, a detailed study of the effect of heliogeomagnetic disturbances on the psycho-emotional state of volunteers has not been carried out; and there is no such study that takes into account the nature of the subjects' choice of coping strategy in stressful situations with differentiation by spheres, their levels of anxiety, and personality type. Previously, we have substantiated in detail the methodological approaches to the choice of methods for assessing the impact of space weather on the psycho-emotional state of volunteers, allowing us to determine the most accurate features of the psycho-emotional status of the subjects.[9]

Purpose of the research**:** To conduct a comparative analysis of the psychological characteristics of the inhabitants of the northern and middle latitudes, depending on their psychological sensitivity to changes in geomagnetic disturbances

Materials and Methods

The study included 78 relatively healthy volunteers (women and men, the average age of 44.2[36.7; 54.3] years) living in the polar (Tiksi), subpolar (Yakutsk) and middle (Saratov) latitudes.

Monitoring of the studied indicators was carried out during March and April 2016.

Inclusion criteria: males and females; relative health at the time of observation.

Exclusion criteria: cognitive impairment of different nature; the use of psychotropic and psychoactive substances; acute forms of disease; chronic diseases in the acute stage; surgical interventions during the last 3 months; cancer pathology; multiple organ failure of different genesis; psychosomatic diseases.

To achieve this goal, we have used the following methods:

- The Spielberger-Khanin scale for reactive (situational) and personal anxiety [10,11]

- E. Heim's technique for revealing individual coping strategies [12,13]

- The projective psycho-geometric test [14,15]

A Spielberger-Khanin questionnaire is focused on the differential measurement of anxiety as a state (reactive or situational anxiety) and personal characteristics (personal anxiety or an anxious disposition).

The E. Heim test allows determining the style of dealing with stress. Heim's technique for psychological diagnosis of coping strategies makes it possible to explore 26 situation-specific options for responding to a complex situation, distributed in accordance with the three main spheres of mental activity: cognitive, emotional and behavioral.

The projective technique, based on a psycho-geometric approach, allows one to instantly determine the form or type of personality and to give a detailed description of the psychological qualities and peculiarities of human behavior in typical situations.

The first part of the Spielberger-Khanin test (to determine reactive anxiety) was presented to the project participants during the entire observation phase. The remaining tests were given once: at the initial stage of the study phase. Every day, during March and April 2016, the Kp-index, an integral indicator of changing geomagnetic disturbance was used.

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

Statistical analysis was performed using StatGraph Plus for Windows 6.0 software package. The normality of distribution of continuous variables was tested by the Kolmogorov-Smirnov test with the Lilliefors correction and Shapiro-Wilk test. Differences of continuous variables departing from the normal distribution were tested by the Mann-Whitney *U*-test. A probability value of $P<0.05$ was considered statistically significant.

Results

Depending on the identified matches, the peak values of the reactive anxiety by Spielberger-Khanin and Kp-index (not less than 60% of cases), all volunteers were divided into 2 groups. Group 1 included persons with similar overlap and, therefore, carriers of psychological sensitivity to changes in the action of heliogeomagnetic factors. Group 2 included persons without a specified sensitivity (i.e., no coincidences).

Results of psychological examination of the inhabitants of the middle latitudes

According to the results of the psychological examination of the inhabitants of the middle latitudes, 44.4% of the observed individuals were included in Group 1 and 55.6% in Group 2. The peculiarities of coping behavior according to the given situation are shown in Figs.1-3. During the Heim test in Group 1, we found that constructive coping strategies were dominant

in the emotional sphere (P=0.04, Fig.2), non-constructive strategies - in the behavioral sphere (P=0.04, Fig.3), and the constructive and non-constructive coping strategies were chosen at approximately the same frequency in the cognitive sphere (Fig. 1). In Group 2, constructive coping strategies prevailed in cognitive (P=0.04, Fig.1) and emotional (P=0.04, Fig.2) spheres; when constructing the actual coping behavior, both constructive and non-constructive options were often used equally (Fig. 3). Our analysis of the choice of coping strategies with differentiation by spheres found that volunteers of Groups 1 and 2 chose, from among the constructive coping strategies in the cognitive sphere, maintaining self–control (33.3% and 33.0%, respectively), cultivating high self-esteem (33.3% and 17.0%, respectively), and problem analysis; the last coping reaction was chosen most often in Group 2 (50.0%). Among the non-constructive coping strategies, ignoring (33.3%), dissimulation (33.3%), and confusion (33.4%) were revealed in individuals of Group 1; Group 2 individuals (100%) preferred to ignore the problems. In the emotional sphere, most of the observed persons were optimistic (Group 2 - 100%, Group 1 – 75%); protest was chosen by 25% of individuals of Group 1. From non-constructive emotional coping reactions, volunteers of Group 1 preferred obedience(100%), and Group 2 individuals – obedience and suppression of emotions (50% and 50%, respectively). Building their own coping behavior, Group 1 individuals, when choosing non-constructive coping strategies, preferred to retreat in the face of difficulties (75,0%) or actively avoid them (25,0%); and choosing constructive strategies, preferred to cooperate with persons who were significant for them (100%). Group 2 individuals, when choosing non-constructive coping strategies, tried to avoid problems (in 100%), and when choosing constructive strategies, cooperated with persons significant for them (66.7%) or turned to them for help (33.3%).

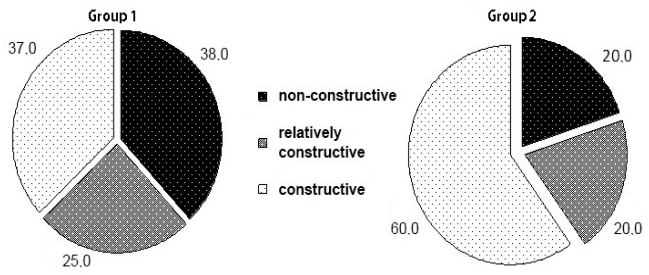

Fig. 1. *Coping strategies in the cognitive sphere in the inhabitants of the middle latitudes (%).*

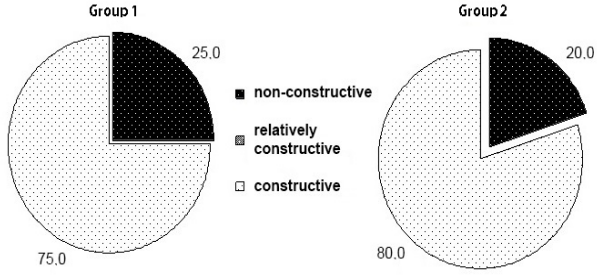

Fig. 2. *Coping strategies in the emotional sphere in the inhabitants of the middle latitudes (%).*

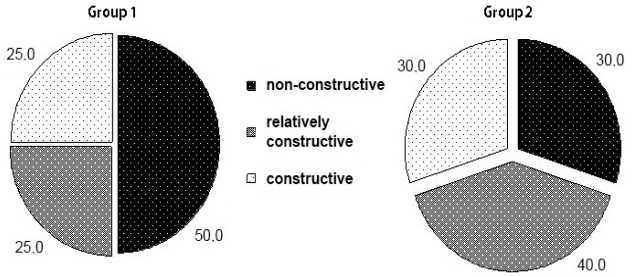

Fig. 3. *Coping strategies in the behavioral sphere in the inhabitants of the middle latitudes (%).*

Psycho-geometric test results with the stimulus material are presented in Figs. 4-5. Thus, Group 1 individuals preferred, among other geometric shapes, a circle (63.0%), rejecting a zigzag (75.0%); Group 2 individuals chose a triangle and a circle (40% in both cases), rejecting the circle and zigzag (30% in both cases) (Figs. 4-5).

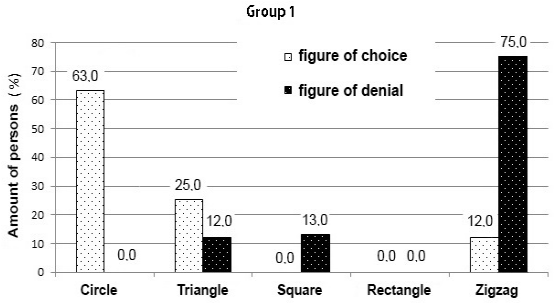

Fig. 4. *Psycho-geometric test results in Group 1 individuals of the middle latitudes.*

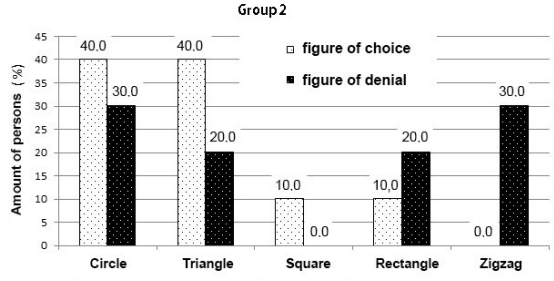

Fig. 5. *Psycho-geometric test results in Group 2 individuals of the middle latitudes.*

Results of psychological examination of the inhabitants of the auroral and sub-auroral latitudes

According to the results of psychological examination of people of the auroral and sub-auroral latitudes, 55.6% of the observed individuals were included in Group 1 and 44.4% in Group 2.

The construction of coping behavior in the cognitive, emotional and behavioral spheres in the inhabitants of the northern latitudes is illustrated in Figs. 6-8. Volunteers of both groups preferred constructive coping reactions in the cognitive sphere (P≤0.05, Fig. 6). In the emotional sphere, constructive

coping strategies were chosen more often in Group 1, and non-constructive strategies in Group 2 (Fig.7). In the behavioral sphere, constructive coping strategies were absent in Group I, and only 9.0% in Group 2; thus, non-constructive strategies were more often chosen in Group 1 than Group 2 ($P \leq 0.04$, Fig. 8).

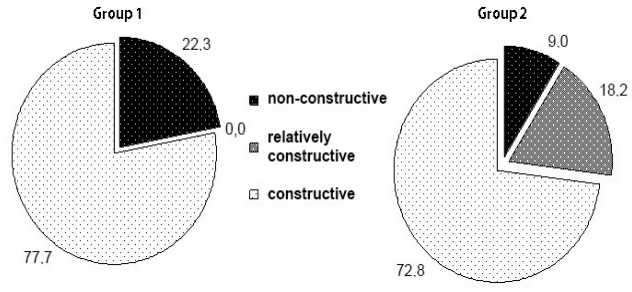

Fig. 6. *Coping strategies in the cognitive sphere in the inhabitants of the northern latitudes (%).*

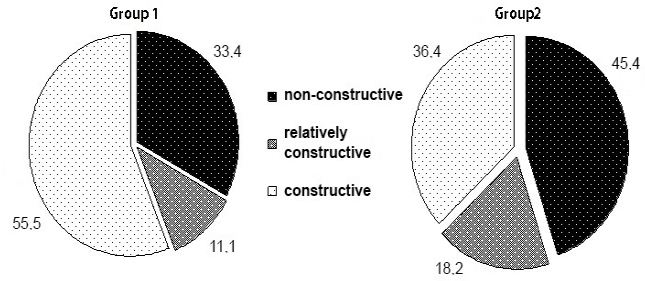

Fig. 7. *Coping strategies in the emotional sphere in the inhabitants of the northern latitudes (%).*

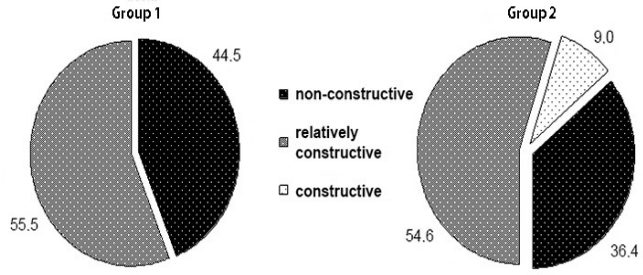

Fig. 8. *Coping strategies in the behavioral sphere in the inhabitants of the northern latitudes (%).*

Our analysis of the choice of coping strategies with differentiation by spheres found that the most common cognitive constructive coping strategies in Group I were maintaining self-control (71.5%) and problem analysis, which was found in 28.5%. In Group 2, on the contrary, problem analysis (87.5%) was more common, and maintaining self-control was found in 12.5%. In volunteers of both groups, optimism prevailed among constructive emotional coping reactions (Group 1 – 100%, Group 2 – 75.0%), and in Group 2, 25.0% of monitoring participants chose protest. Of the non-constructive emotional coping styles, representatives of both groups preferred suppression of emotion (Group 1 – 66.7%, Group 2 – 100%). At the same time, Group 1 individuals, in some cases, showed aggression (33.3%). In the behavioral sphere, constructive coping strategies were absent in Group 1; at the same time, Group 2 persons used altruism and care-seeking equally. As for non-constructive behavioral coping strategies, all persons of Group 1 used active avoidance, and persons of Group 2, in most cases, chose retreat (75.0%), and less often, active avoidance (25.0%).

In the psycho-geometric test, Group 1 individuals preferred, among other geometric shapes, a circle and a triangle (33.3% in both cases), rejecting a zigzag (55.5%) (Fig. 9). Group 2 persons preferred the square and triangle (40.0% and 30.0%, respectively), also rejecting a zigzag (70.0%) (Fig. 10).

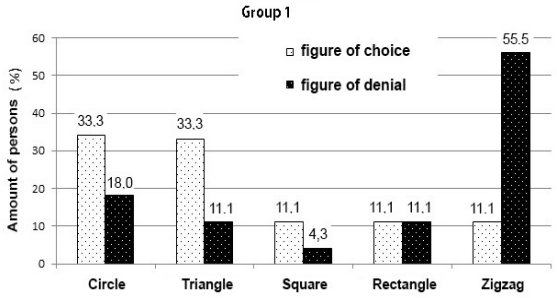

Fig. 9. *Psycho-geometric test results in Group 1 individuals of the northern latitudes.*

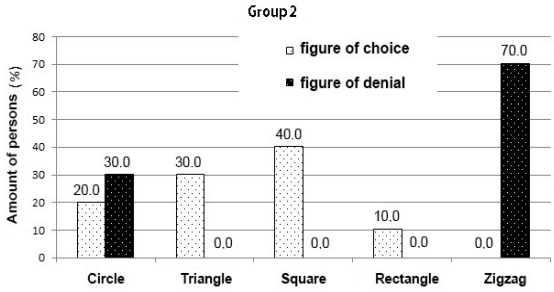

Fig. 10. *Psycho-geometric test results in Group 2 individuals of the northern latitudes.*

Discussion

Based on the results obtained, psychological portraits of the inhabitants of the middle latitudes, depending on the presence or absence of their psychological sensitivity to the actions of heliogeomagnetic factors, can be presented as follows: Group 1 representatives, facing a stressful situation and building their coping behavior, are the most constructive in the emotional sphere: they are optimistic, friendly, do not tend to suppress either their positive emotions or negative emotions, and are ready to protest, if necessary. Group 2 representatives are also the most constructive in the emotional sphere, as optimistic as persons from Group I, but unlike the latter, tend to suppress their emotions without expressing active protest against negative circumstances.

Representatives from both groups living in the middle latitudes, in some cases, can be submissive under the influence

of negative factors, but only persons of Group 1 are able to protest against hostile circumstances, while Group 2 persons suppress their emotions.

In the cognitive sphere, representatives of Group 2 turned out to be more constructive, preferring problem analysis. Representatives of Group 1 also, although to a lesser extent, are able to pay attention to assessing cause-effect relationships and emerging problems, along with maintaining self-control and self-esteem.

In both groups, the most problematic coping sphere among residents of middle latitudes was the behavioral sphere, which most clearly manifested in Group 1. Group 1 persons are prone to retreat and less likely to avoid problems, while Group 2 persons always actively avoid problems and difficulties.

The constructive behavior of the representatives of both groups living in the middle latitudes is mainly expressed in cooperation with persons of importance to them.

The above-mentioned psychological characteristics of Group 1 individuals can be complemented with their sociability, delicacy, friendliness, hospitality, flexibility and the desire to avoid conflicts (mainly the choice of the circle and the denial of the zigzag at psychosomatic testing).

In Group 2 individuals, purposefulness, leadership traits, activity, and sometimes conflictedness, as well as contradictory personality traits, can serve as a supplement to their psychological portrait.

The inhabitants of the northern latitudes—representatives of both groups—are the most constructive in the cognitive sphere. Self-control and, in part, the desire to analyze the problems were dominant in Group 1. In Group 2, the dominant coping cognition was a problem analysis and, to a much lesser extent, maintaining self–control. In the emotional sphere, the representatives of Group 1 were more constructive than in Group 2. At the same time, optimism dominated in both groups, especially in Group I, and the representatives of Group 2 sometimes protested against negative circumstances. Quite often in Group I and always in Group 2, the inhabitants of polar and subpolar latitudes suppressed their emotions, and Group 1 volunteers, from time to time, experienced aggression. In the representatives of both groups, the most unfavorable situation was in the construction of the actual coping behavior: There were no constructive behavioral coping strategies completely in Group 1 (these persons preferred to avoid problems exclusively); there were few constructive coping strategies in Group 2, and their non-constructive behavior was represented mainly by retreat and, in part, active avoidance.

The personality traits of Group 1 individuals can be complemented with their sociability, responsibility, purposefulness, concentration and lack of contradiction of psychological characteristics. In Group 2, those traits are restraint, propensity to individual activities, logical reasoning, diligence, conservatism, and conformity.

Conclusion

Thus, in 2016, in the northern and middle latitudes, the distribution of persons who were psychologically sensitive and insensitive to changes in heliogeomagnetic factors did not differ significantly and was approximately equal. In the course of the study, both coinciding and latitude-related differences in the psychological characteristics of different groups of volunteers were established.

In the inhabitants of the polar and subpolar latitudes, especially in Group 1, the behavioral sphere was the most vulnerable (no constructive coping strategies). In addition, their changes in the emotional sphere contributed to the manifestation of aggressiveness and suppression of emotions against the background of general concentration, purposefulness and integrity of the individual. Regardless of the latitude of residence, volunteers, psychologically sensitive to changes in heliogeomagnetic factors, were more constructive in the emotional sphere, and the least in the behavioral, which gives reason to state that, depending on the factors of space weather, the behavior of the individual is the most vulnerable.

Conflict of interest

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Dermatology

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Morphological Substantiation of Clinical Efficacy of Platelet Rich Plasma in the Treatment of Androgenetic Alopecia

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Abstract

The aim of the study was morphological substantiation of clinical efficacy of platelet rich plasma (PRP) in the treatment of patients with androgenetic alopecia (AGA).

Materials and Methods: The study included 25 men aged from 20 to 43 years. AGA degree II-IV on the Hamilton-Norwood scale was diagnosed in 23 (92%) patients and degree I in 2 patients. All patients received intradermal injections of PRP. The course of treatment consisted of 4 procedures with an interval of 4 weeks between each procedure. The clinical efficacy of the therapy was evaluated by the dynamics of morphometric indicators of hair growth. The morphological analysis included an assessment of indicators in horizontal sections and was carried out at 4 levels: at the level of subcutaneous adipose tissue, sweat glands, sebaceous glands, and mouths of hair follicle (HF). Sections were stained with H&E. Histological examination was performed before and after treatment. Against the background of treatment with PRP, hair density increased by 12% (P=0.000), average hair diameter by 12% (P=0.002), and the share of vellus and telogen hair decreased by 17% (P=0.002) and 16% (P=0.028), respectively. At the same time, the amount of anagen hair in relation to telogen hair increased by 18% (P=0.016). Histologically, the increase in hair density was accompanied by a significant increase in the number of HF at the mouth level (Δ+96%, P=0.004) and at the level of sweat glands (Δ+54%, P=0.037), as well as a tendency for their number to increase at the level of the sebaceous glands. These increases were combined with a significant decrease in the proportion of telogen hair (Δ-43%, P=0.023) and vellus hair (Δ-29%, P=0.037).

Conclusion: The positive clinical effect of PRP therapy is due to significant morphofunctional changes in hair follicles. (**International Journal of Biomedicine. 2018;8(4):317-320.**)

Key Words: androgenetic alopecia • hair follicle • platelet rich plasma • treatment • morphological substantiation

Introduction

Platelet rich plasma (PRP) is a very promising method for the treatment of androgenetic alopecia (AGA).[(1)] Z.J. Li et al.[(2)] indicated that PRP prolongs the anagen phase, promotes cell proliferation and lifespan of the dermal papilla during the hair growth cycle, and increases the cell survival time by inhibiting apoptosis. Clinically, this is manifested in a decrease in the intensity of hair loss (telogen fraction). It is believed that the growth factors contained in platelets bind to receptors sensitive to them and activate the proliferative phase of the hair.[(3)] Gkini Maria-Angeliki et al.[(4)] conducted a systematic review of 14 published studies on the use of PRP in the treatment of AGA, among which the main clinical results were an increase in density and a decrease in hair loss. The accumulated world experience in the use of PRP-therapy in the treatment of AGA is mainly clinical. Only scarce data are available on the analysis of the dynamics of hair growth indicators in accordance with the standards of morphological diagnosis on the background of PRP-therapy.

The aim of the study was morphological substantiation of clinical efficacy of PRP in the treatment of patients with AGA.

Materials and Methods

The study included 25 men aged from 20 to 43 years (mean age, 30.0±2.5 years). AGA degree II-IV on the Hamilton-Norwood scale[(4)] was diagnosed in 23 (92%) patients and degree I in 2 patients. The average disease duration was 3.4±0.97 years. To obtain PRP, blood samples (18 ml) were collected from each

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patient by venipuncture into 2 tubes with an anticoagulant. As an anticoagulant, 3.8% sodium citrate was used in a ratio of 1:9. Centrifugation was carried out twice. The first centrifugation was carried out at 1800 rpm for 5 min, after which the erythrocyte mass was separated. Plasma containing leukocyte and platelet layers was subjected to a second centrifugation at 2500 rpm for 10 min. As PRP, 2 ml of the lower part of the supernatant from each tube was used. The plasma was separated manually. An official calcium chloride solution was used as an activator in a ratio of 1:20. The surface of the scalp was treated with a chlorhexidine solution; local anesthesia was not applied. The resulting PRP was injected intradermally into the scalp by microinjection, at approximately 0.15 ml per injection. The course of treatment consisted of 4 procedures with an interval of 4 weeks between each procedure. Written informed consent was obtained from each patient.

The clinical efficacy of the therapy was evaluated by the dynamics of morphometric indicators of hair growth. A trichological study was performed using a digital video camera (Aram Huvis Co., Ltd; Korea) and the TrichoSciencePro v1.3 software. The density of hair was determined on 1 cm^2—the share of vellus and telogen hair - in %, the average diameter of all hair - in microns, and the ratio of hair in the telogen/anagen stage, as the amount of anagen hair per 1 telogen hair. The control points were marked with a tattoo mark and were in the parietal zone, approximately 2 cm closer to the center from the border of the thinning hair.

Skin biopsies for histological examination were obtained from 12 patients before and after treatment. Skin biopsies were performed under local anesthesia with a 1% lidocaine solution with epinephrine. The material was sampled by a punch for biopsy with a diameter of 4 mm.[5,6] Skin pieces were fixed in 10% neutral buffered formalin, dehydrated in alcohols of increasing concentration and embedded in paraffin according to the standard procedure. Sections were stained with H&E.

The morphological analysis included an assessment of indicators in horizontal sections and was carried out at 4 levels: at the level of subcutaneous adipose tissue, sweat glands, sebaceous glands, and mouths of hair follicle (HF). Hair density was evaluated at all levels. The diameter of the hair shafts and the number of vellus and miniaturized hair were calculated at the level of the sebaceous glands of HF. Hair was considered to be vellus if the core diameter was equal to or less than the thickness of the inner shell of the follicle and was ≤0.03 mm. Hair in the anagen, catagen and telogen stages was also counted at the level of the sebaceous glands. The telogenous hair was classified as hair without an inner shell or at the late telogen stage, as well as with irregular accumulation of basaloid star-shaped cells in the form of islands with nuclei located on the periphery. Hair characterized by the presence of the inner sheath of the hair root and the absence of cell necrosis was considered to be in the anagen phase. Histological examination was performed using the 3DHistech Pannoramic scanning system and the Pannoramic Viewer 1.15.4 v. software (3DHISTECH Ltd., Hungary).

Statistical processing and visualization of the results was carried out using Microsoft Office Excel (version 15.0.50.31.1000, 2013) and standard R programming language tools (version 3.4.37). 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. The Wilcoxon criterion was used to compare the differences between the paired samples. A probability value of $P<0.05$ was considered statistically significant.

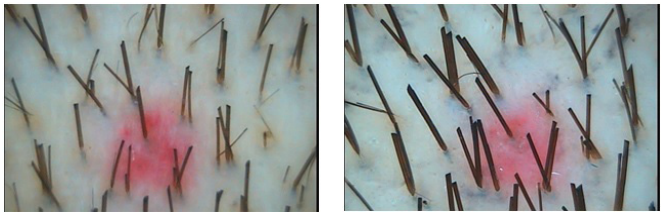
Results

Against the background of treatment with PRP, all hair growth indicators underwent significant changes. Hair density increased by 12% ($P=0.000$), average hair diameter by 12% ($P=0.002$), and the share of vellus and telogen hair decreased by 17% ($P=0.002$) and 16% ($P=0.028$), respectively. At the same time, the amount of anagen hair in relation to telogen hair increased by 18% ($P=0.016$) (Table 1, Figure 1).

Table 1.

Dynamics of morphometric indicators of hair growth

Variable	Before treatment	After treatment	Dynamics of change		*P*-value
			Abs.	Δ (%)	
Hair density on 1cm^2	381.5±45.4	426.1±50.1	44.6	12%	0.000
Share of vellus hair, %	49.6±7.3	41.0±7.7	-8.6	-17%	0.002
Average diameter of all hair, μm	39.8±3.5	44.4±4.5	4.6	12%	0.002
Share of telogen hair, %	42.0±6.4	35.3±7.0	-6.8	-16%	0.028
Ratio of hair in the telogen/ anagen stage	1/2.28±1/1.51	1/2.68±1/0.88	1/0.40	18%	0.016



(a) (b)

Fig.1. *The patient who received PRP injections: (a) - before treatment; (b) - after treatment. Phototrichogram, magnification ×60.*

The increase in hair density was accompanied by a significant increase in the number of HF at the mouth level (Δ+96%, $P=0.004$) and at the level of sweat glands (Δ+54%, $P=0.037$), as well as a tendency for their number to increase at the level of the sebaceous glands. These increases were combined with a significant decrease in the proportion of telogen hair (Δ-43%, $P=0.023$) and vellus hair (Δ-29%, $P=0.037$) (Table 2, Figure 2).

Table 2.

The morphological analysis of indicators in horizontal sections

Variable	Before treatment	After treatment	Dynamics of change		*P*-value
			Abs.	Δ (%)	
The level of subcutaneous adipose tissue					
HF number	29.7±23.0	57.0±9.5	27.3	92%	0.087
The level of sweat glands					
HF number	44.0±16.9	68.0±14.9	24.0	54%	0.037
The level of the sebaceous glands					
HF number	67.2±21.9	92.0±32.0	24.8	37%	0.057
Share of vellus hair, %	38.2±8.2	26.8±7.6	-11.4	-29%	0.037
Average diameter of all hair, μm	38.7±4.8	45.0±12.1	6.3	16%	0.202
Share of telogen hair, %	25.4±5.3	14.6±8.4	-10.8	-43%	0.023
The mouth level					
HF number	77.1±10,3	151.0±68.0	73.9	96%	0.004

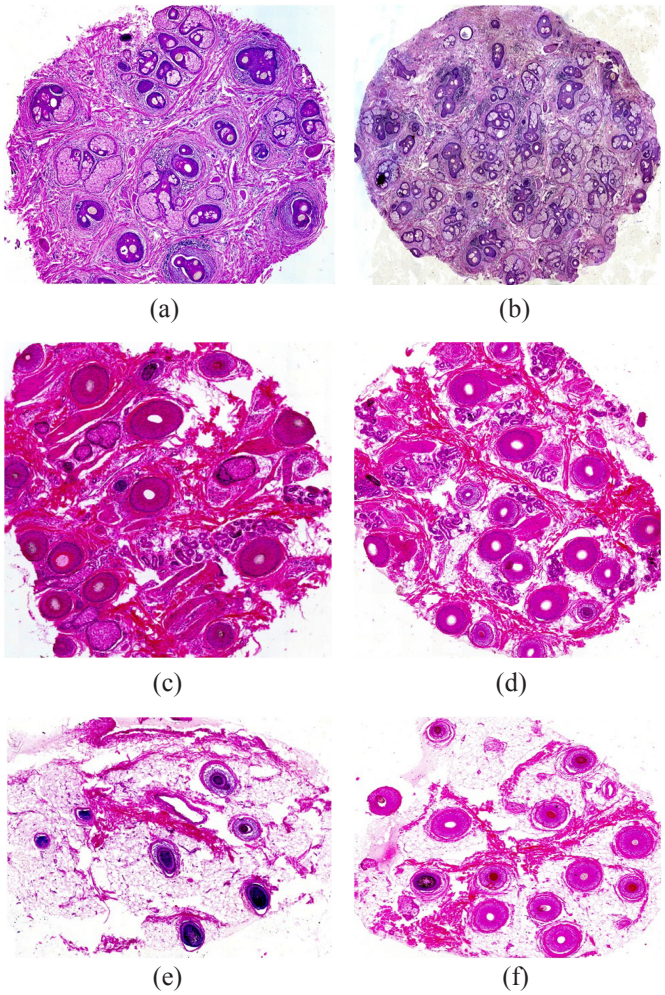


Fig. 2. *Histological examination: horizontal sections, H&E staining. The number of HF at the mouth level (×60) before (a) and after treatment (b), at the level of sweat glands (×100) before (c) and after treatment (d), and at the level of the sebaceous glands (×100) before (e) and after treatments (f).*

Discussion

Hair loss has a significant effect on the psycho-physiological state and is associated with low self-esteem and depression of patients. The treatment of AGA is limited and includes topical administration of minoxidil, oral finasteride (FDA approved), both as monotherapy and as a combination.

The regenerative potential of PRP has attracted the attention of doctors of many specialties, including trichology. It is known that growth factors activate the proliferative phase and transdifferentiation of hair and stem cells, and the proliferation of dermal papilla cells of HF, and thus play a key role in the growth of the hair shaft. In 2018, PRP-therapy was given attention in the European guidelines for the treatment of AGA, while it was recognized that clinical data are still insufficient for a good evidence base of this treatment method. The availability of a support protocol regarding the preparation of PRP, the frequency of use and the amount of plasma injected remain topical issues.

We prepared PRP by the double spinning method with "soft spin," in which the layers of blood cells were separated manually. We also applied platelet activation through coagulation, which causes the secretion of various growth factors and potentiates mitogenic effects in different cell types. Activated PRP promotes the proliferation of dermal papillary cells and prevents their apoptosis.

The course of our study demonstrated a significant increase in the density and average diameter of hair and a decrease in the share of vellus and telogen hair. This was accompanied by a significant increase in the number of HF at the level of mouths and sweat glands, as well as a tendency to increase them at the level of the sebaceous glands.

The decrease in the proportion of telogen and vellus hair was significant, both in clinical and histological data. The increase in the diameter of the rods, according to the phototrichogram, was significant, but it was not significant histologically. It is possible that some difference in indicators is related to different levels of counting: the phototrichogram shows the condition of the hair on the surface of the scalp, which, when morphologically examined, corresponds to the level of the HF mouth. Morphological analysis was carried out a little deeper, at the level of the sebaceous glands.

The results of our study are consistent with data published in the world literature on the effectiveness of PRP. A comparative analysis of the phototrichological and morphological results of treatment confirmed positive unidirectional dynamics.

Our study has some limitations. During the trichoscopic evaluation, objective results were obtained, but the sample size for the morphological analysis was small. Average patient follow-up time was also too short to show the long-term effectiveness of the treatment. Thus, further studies with longer observation and with a large number of samples are needed.

Conclusion

PRP injections are a simple, effective treatment for hair loss, and can be considered a valuable treatment for AGA.

The positive clinical effect of PRP therapy is due to significant morphofunctional changes in hair follicles. These effects of PRP on HF are possible under the condition that pathological processes are reversible and are not specific for AGA, which makes further research relevant to the possible effect of PRP on the expression of specific proteins that trigger pathological signaling pathways.

Conflict of interest

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Ultrasound Imaging

INTERNATIONAL JOURNAL OF BIOMEDICINE

Ultrasound Assessment of Cervical Length in the First Trimester of Pregnancy to Predict Preterm Birth

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Abstract

The aim of this study was to examine the potential value of routine measurement of cervical length (CL) in singleton pregnancy at 11 to 14 weeks of gestation for predicting the risk for spontaneous preterm delivery (PD).

Materials and Methods: CL was assessed using transvaginal sonography (TVS) in the gestational period between 11 and 14 week in 1517 women. Childbirth at 22-36 weeks was considered as PD. The control group included pregnant women who gave birth within 37 weeks or more.

Results: The mean age of mothers was 25.3±4.9 years (age range of 17 to 43). Among them, 846 (55.8%) women were primiparous and 671 (44.2%) - multiparous. The average CL was 38.9±4.3 mm (range of 18 to 49 mm). The area under the ROC curve of the corresponding relationship between the CL forecast and the probability of PD occurrence was 0.84. When selecting the threshold value of the function at the point 35 mm, we predicted a high risk of PD with a sensitivity of 66.2% and a specificity of 84.2%. At the CL value of 30 mm, the sensitivity of the method was 51.5%, specificity 98.7%. For CL: OR=0.79, 95% CI: 0.75-0.83; *P*=0.0001. Thus, the most optimal cut-off CL is the threshold value of 30 mm.

Conclusion: TVS is an objective, reproducible and reliable method for assessing the cervix uteri and can predict the risk of preterm delivery.(**International Journal of Biomedicine. 2018;8(4):321-323.**)

Key Words: singleton pregnancy • cervical length • preterm delivery • transvaginal sonography

Abbreviations

CL, cervical length; **PD**, preterm delivery; **ROC** curve, Receiver Operating Characteristic curve; **TVS**, transvaginal sonography

Introduction

Preterm delivery (PD) remains a significant obstetric problem, determining the rate of perinatal mortality.[1] Proven predictors of PD are PD in history, the level of fetal fibronectin, and shortening of cervical length (CL).[2,3] Fibronectin, as a risk marker, is not used in Russia. The study of CL and the search for new markers indicate real prospects for medical interventions. Numerous studies have shown the relationship between CL in the second trimester of pregnancy (18 to 22 weeks) and PD.[4-7] CL<30 mm is considered critical up to 20 weeks, and less than 20 mm is an absolute criterion of cervical insufficiency in any term.[3,8] Early prevention of PD is associated with progesterone therapy in early pregnancy, which requires the development of criteria for evaluation of CL in the first trimester.[9,10]

The aim of this study was to examine the potential value of routine measurement of CL in singleton pregnancy at 11 to 14 weeks of gestation for predicting the risk for spontaneous PD.

Materials and Methods

A prospective observational study was conducted in the period from April 1, 2012 to September 30, 2013 in the Tula Regional Perinatal Center as part of a screening program for chromosomal and structural abnormalities.

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Inclusion criteria were 11 to 14 weeks of gestation, singleton pregnancy, and no complaints at the time of examination.

Exclusion criteria were surgical treatment of the cervix, multiple pregnancy, congenital anomalies of the female genital tract, and fetal abnormalities.

Description of medical intervention

The linear dimensions of the cervix were estimated as the distance from the projection of the internal orifice to the projection of the external orifice in sagittal scanning, taking into account the curvature of the cervical canal. Tracing was used to measure the length of the cervical canal.

A total of 1637 women met the inclusion criteria. CL was assessed in the gestational period between 11 and 14 weeks. Out of 1637 women, 112 were lost for follow-up, and 8 women underwent cesarean delivery because of an emergency with the fetus. Therefore, 1517 women were included for further examination. Childbirth at 22-36 weeks was considered as PD. The control group included pregnant women who gave birth within 37 weeks or more. Gestational age was determined by the data of the first day of the last menstrual period and ultrasound data on crown-rump length. TVS was performed using a 7.5-MHz transvaginal probe (MEDISON V20). The study was carried out by the doctor-expert, certified by the Fetal Medicine Foundation for the first trimester screening and CL assessment.

Statistical analysis was performed using IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.). Correlation analysis, nonparametric quantitative data analysis, binary logistic regression with determination of predictive model sensitivity and specificity, and evaluation of diagnostic significance using ROC-curves method were used. A probability value of $P<0.05$ was considered statistically significant.

Results

The mean age of mothers was 25.3±4.9 years (age range of 17 to 43). Among them, 846 (55.8%) women were primiparous and 671 (44.2%) - multiparous. The average CL was 38.9±4.3 mm (range of 18 to 49 mm). The data of CL distribution do not follow a normal distribution according to the Kolmogorov-Smirnov normality test (Figure 1).

The correlation analysis showed a correlation between CL and PD (r=+0.321). The resulting model accounted for 10.3% of the factors determining changes in the term of labor (Figure 2).

Binary logistic regression with determination of sensitivity and specificity of the prognostic model was used to estimate the probability of PD occurrence depending on the quantitative index of CL. The observed dependence is described by the equation:

$$p = \frac{1}{1+e^{-z}}$$

$$z = 10.31 - 0.374*X$$

where p is the probability of PD occurrence
X-CL measured in the first trimester of pregnancy (mm)
10.31 – the estimated constant

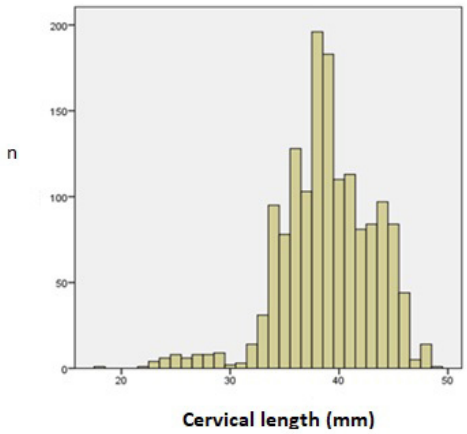

Fig. 1. *CL distribution at 11-14 weeks of gestation in 1517 singleton pregnancies.*

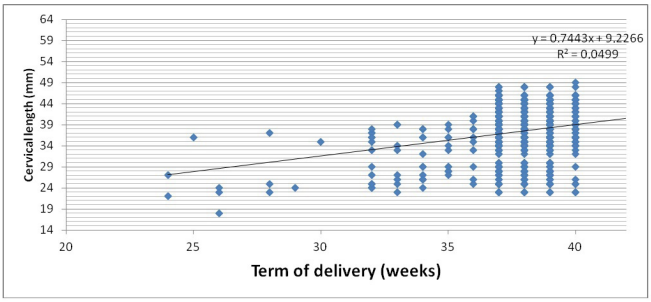

Fig. 2. *Dependence of the labor term on CL in the first trimester.*

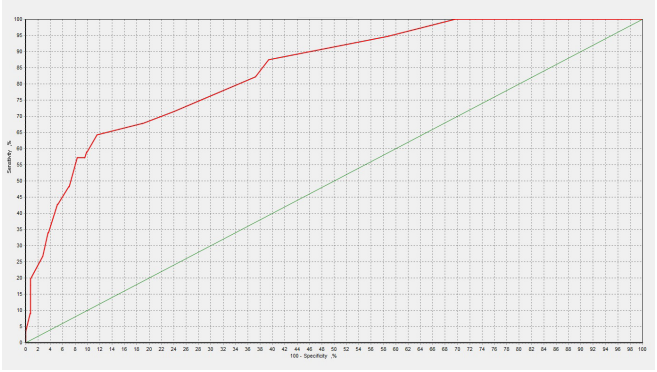

Fig. 3. *ROC- analysis: CL index measured in the first trimester of pregnancy for PD prognosis.*

Based on the values of regression coefficients, the factor CL, measured in the first trimester of pregnancy, has a feedback with the probability of PD: With a decrease in CL, the probability of delivery before 37 weeks increases.

The resulting regression model is statistically significant ($P<0.001$). Based on the value of the coefficient of determination (Nagelkerke's R^2) the model (1) takes into account 39.3% of the factors determining the probability of PD. This model has a diagnostic efficiency of 96%, specificity of 99.4%, but rather low sensitivity of 38.2%.

Evaluation of diagnostic significance of the CL index measured in the first trimester of pregnancy for PD prognosis was performed by ROC-analysis (Figure 3).

So, it is necessary to find the optimal separating value CL (cut-off), which allows classification of patients according to the degree of PD risk, which has the best combination of sensitivity and specificity.

The area under the ROC curve of the corresponding relationship between the CL forecast and the probability of PD occurrence was 0.84. When selecting the threshold value of the function at the point 35mm, we predicted a high risk of PD with a sensitivity of 66.2% and a specificity of 84.2%. At the CL value of 30 mm, the sensitivity of the method was 51.5%, specificity 98.7%. For CL: OR=0.79, 95% CI: 0.75-0.83; *P*=0.0001.

Discussion

Despite the lack of effective measures to prevent preterm labor, identification of individuals at high risk for PD remains important for gaining an understanding of the various pathophysiological pathways and for assessment of therapeutic efficacy.[11] Although preterm labor can be the result of various causes,[12,13] cervical shortening has consistently been shown to occur prior to the onset of preterm labor.[2,6] The effectiveness of measures aimed at prolongation of pregnancy depends on the obstetric situation, pregnancy term and fetal prognosis.[9] This makes it necessary to identify women at risk for PB as early as possible. TVS is an objective, reproducible and reliable method for assessing the cervix uteri and can predict the risk of PD.[14] Assessment of the cervix enables measurement of CL.

There is an inverse correlation between CL and the date of birth. The high specificity of the method allows us to avoid unnecessary interventions, such as tocolysis or cervical cerclage, in uncomplicated pregnancies. The low sensitivity of the method should be noted: with CL=35 mm - 38.2%, CL=30 mm - 48.5%, and CL=25 mm -76.5%. Although, CL≤25 mm is associated with a significantly increased frequency of PD, detecting such values in the first trimester of pregnancy is extremely rare. At this time it seems to us that the most optimal cut-off CL is the threshold value of 30 mm. High specificity will exclude women with normal risk, and to offer those who are in the risk zone additional cervicometry in 16 weeks and a subsequent decision on the need for the use or cancellation of progesterone drugs or the transvaginal cervico-isthmic cerclage in uncomplicated pregnancies. To further improve the forecasting model, using the latest knowledge of the development model and potential risk factors, it is necessary to contribute to the personal risk assessment of spontaneous preterm delivery.[15]

Conflict of interest

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Ultrasound Imaging

INTERNATIONAL JOURNAL OF BIOMEDICINE

Ultrasound of Acute Appendicitis in the Admission Room of a Multidisciplinary Surgical Hospital

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Abstract

Ultrasound (US) for diagnosing acute appendicitis and its complications in the admission room may be used as a primary diagnostic method in urgent patients. A total of 180 adult patients underwent US for suspected appendicitis; these results showed high possibilities in pathology screening and differential diagnosis from similar clinical diseases. The accuracy of US in detecting acute appendicitis with obvious clinical findings and typical position is 100%, but in retrocecal and retroperitoneal forms, it decreases to 84%. With our algorithm of the right iliac and localized pain regions, US scanning optimizes the screening of patients with suspected AA presented in the admission room. (**International Journal of Biomedicine. 2018;8(4):324-326.**)

Key Words: appendix • acute appendicitis • ultrasound • diagnostic algorithm

Introduction

The optimization of the diagnostic algorithm of acute appendicitis (AA) remains a serious problem. This is the most frequent pathology in urgent surgery: it amounts to 80%-85% of emergency procedures. The prevalence of this disease is estimated to be 5 persons per 1000 annually.[1,2] Misdiagnosing appendicitis leads to unnecessary surgery and excision of unaltered appendices in about 15% of cases, despite the obvious clinical manifestations.[1,3] This raises the necessity of imaging methods that could increase the diagnostic accuracy of the appendiceal destruction stage in the admission room, differentiate it from other diseases, select the surgical approach, avoid harming a patient with an unnecessary intervention and reduce the diagnostic time.

Since 1980, ultrasonography has been a useful method to visualize the altered appendix, and in 1986, it became the main accessory modality with an adjacent compression technique during abdominal examination. Many surgeons emphasize the importance of imaging methods only in situations where no more exact clinical symptoms exist. If the clinical presentation and the anamnesis are clear, the diagnosis of AA is established without extra diagnostic methods.[1,2,4,5]

According to a number of authors, the US accuracy for this kind of pathology varies from 66% to 100%.[2,3,6] The disadvantages of US are operator-dependence and the factors that complicate the visualization of the appendix, such as obesity, hyperpneumatisation of bowel or inadequate transducer compression. There is a need for a scanner of expert class at the stage of the admission room of a multidisciplinary hospital because the technical data are extremely important in abdominal ultrasound examination.[1,2,7] Moreover, the differentiation of AA types and their complication facilitates the choice of surgical approach and patient treatment.

The purpose of our study was to estimate the possibilities and the diagnostic efficacy of US examination of patients with suspected AA in the admission room.

Materials and Methods

We analyzed the sonographic data of 180 patients between 18 and 78 years of age (mean age, 25±4 years)

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who presented to the admission room of Municipal Clinical Hospital, named after S.I. Spasokukotsky, from September 2017 to September 2018 with suspected AA. The clinical symptoms of this disease and its sonographic signs were considered. The clinical examination in a surgeon's cabinet at the admission room was in the first stage; the second one included the US examination of abdominal, retroperitoneal, and pelvic areas focused on the right side of iliac region. Ultrasound was performed with a Sonoscape S20 (SonoScape, China) with different types of modes and probes according to the patient's physique, mass of subcutaneous tissue, intestinal pneumatosis and imaging quality. If the US findings of AA were absent, we used abdominal computed tomography (CT) in the surgical department. All findings were confirmed by surgical intervention or CT if there were no signs of AA. We accord high priority to the diagnostic accuracy at the stage of the admission room as the most important place for medical sorting in the multidisciplinary hospital.

Results

From 180 patients who presented to the admission room with suspected AA, the US examination confirmed this diagnosis in 122 (68%) cases. It was impossible to form a diagnosis with US in 18 (10%) patients due to an atypical appendix position and some constitutional features, but in 16 cases out of the 122, US showed inflammation symptoms, such as paracolic consolidation, bowel wall thickening in the right side of the iliac region, and peritoneal effusion confirmed on CT. The presence of an unaltered appendix or other pelvic and retroperitoneal pathology (also confirmed on CT) refuted the diagnosis of AA in 40 (22%) patients; 4 (10%) patients from this group were found to have an unaltered appendix in the typical position.

One hundred and forty patients with a confirmed AA had different appendix positions (Table 1):

• Typical [105 (75%)] – the inflammatory changes were detected on US in 100%

• Retrocecal and retroperitoneal [19 (14%)] – in 11 (58%) patients the diagnosis was confirmed on US, 8 (42%) patients underwent CT due to absence of clear features of AA on US

• Ascending [7 (5%)] – detected on US in 6 (86%) patients

• Medial [9 (6%)] position could not be visualized on US because of atypical position and unrepresentative clinical symptoms (differential diagnosis included acute pancreatitis and mesenterial thrombosis)

Table 1.

Diagnostic accuracy of US in detecting AA at the admission room stage

Appendix positions	Found		Not found	
	Total	%	Total	%
Typical	105	100	0	0
Retrocecal and retroperitoneal	11	58	8	42
Ascending	6	86	1	14
Medial and other atypical variants	0	0	9	100

From a sufficient number of results, we can conclude that the efficiency of US in detecting an appendix in its typical position was 100%; in retrocecal and retroperitoneal types, it was possible to visualize the appendix in over 50%. It was not practical to form a diagnosis in medial and retroperitoneal positions at the stage of the admission room due to a time limit, absence of patient preparation for examination, or atypical clinical presentation mimicking another acute surgical disease (Table 2). It was found that the specificity in detecting AA by ultrasound was 100%; the accuracy rate was also high – the absence of false-positive results proved this fact. We confirmed a diagnosis of AA only if the appendix was obviously visualized to reduce the diagnostic time and to avoid unnecessary examinations that could increase time to surgical intervention.

Table 2.

Diagnostic efficacy of US in detecting appendix in different positions

Appendiceal positions	Sensitivity,%	Specificity,%	Accuracy, %
Typical	100	100	100
Retrocecal and retroperitoneal	58	100	88
Ascending	86	100	98
Medial and other atypical variants	0	100	84

The US diagnostic algorithm of patients presented to the admission room with suspected AA was completed. In cases of typical clinical findings, the right iliac region should be carefully examined: firstly, we visualized the head of the caecum and the possible changes in tissue echogenicity, wall thickness, the number of layers, and the presence of effusion and infiltration. Further, we estimated the appendix: its diameter (>6 mm – inflamed), wall thickness (>1.5 mm – inflamed) and echogenicity, and the presence of fluid or inclusions into the appendiceal lumen (Figure 1).

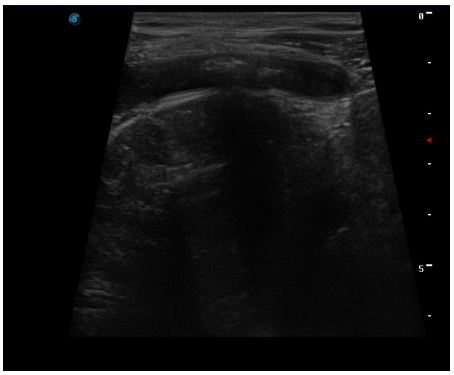

Fig. 1.

US. Inflamed appendix in typical position: diameter of 8-9 mm, hyperechogenicity and irregular wall with thickness of 2 mm, fluid into appendiceal lumen.

If there are some clinical findings but there is no appendix in the typical position when a patient is lying on

his back, he should be turned on his left side to exclude the retrocecal appendiceal position (Figure 2).

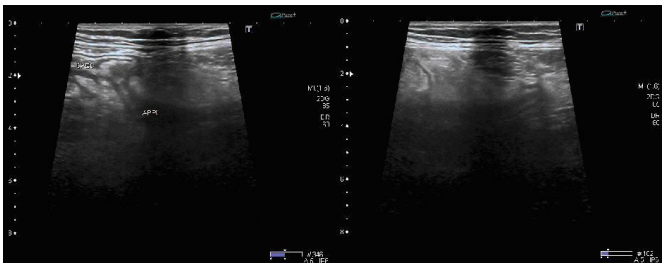

Fig. 2.

US. Inflamed appendix in retrocecal position. Appendix is located behind the bowel loop, appendiceal diameter is 8 mm; hyperechogenic walls with thickness of 2.5 mm. Fluid into appendiceal lumen, peri-appendicular infiltration. Appendiceal orifice and its origin from the cecal head are shown.

The changes of echogenicity and wall thickness of the appendix permit us to define a type of AA and its complications and, consequently, to choose a surgical approach (Figure 3 and 4).

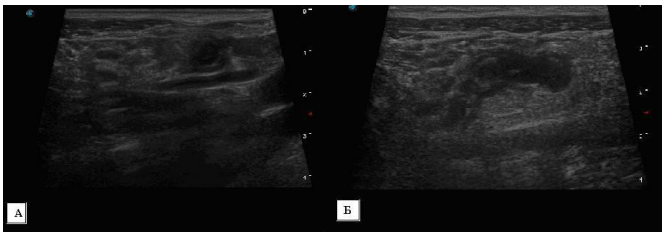

Fig. 3.

US. A – phlegmonous appendicitis: target-sign, hyperechogenicity, wall thickness *of 2-2.5 mm, fluid into appendiceal lumen. B – gangrenous appendicitis: erased margins, absence of layers differentiation in middle and upper thirds of appendix without target-sign.*

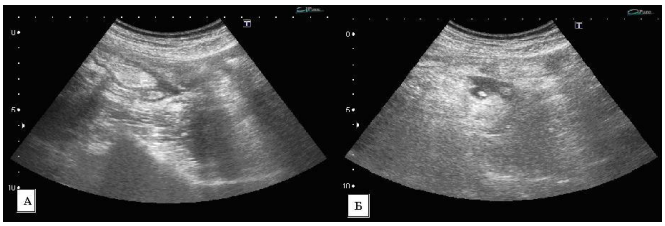

Fig. 4.

Perforation of destructive phlegmonous appendicitis with periappendicular infiltration and abscess in the apex. A – inflamed appendix with periappendicular infiltration, hyperechogenic walls with thickness of 2-2.5 mm and fluid collection in the apex. B – heterogeneous fluid collection with irregular blurred margins and hyperechogenic inclusions (abscess).

Conclusion

Ultrasound showed a high efficacy in detecting acute appendicitis, revealed the inflammation findings and appendiceal destructive changes, and provided the differential diagnosis to clinically similar diseases. US allows us to confirm the reliable diagnosis in cases of appendiceal accessible position and exact clinical assignment, even if the patient flow is high.

• The accuracy of ultrasound in detecting appendicitis with obvious clinical findings and typical position is 100%, but in retrocecal and retroperitoneal forms, it decreases to 84%.

• Ultrasound is a prime modality for the mass examination of patients presented to the admission room in view of the absence of a radiation dose and high imaging quality using a US-system of expert class.

• Absence of ultrasound findings of acute appendicitis mostly confirms absence of appendiceal inflammation.

• Computed tomography should be used if the clinical presentation differs from ultrasound findings.

• Ultrasound of the right iliac and localized pain regions should be implemented into daily practice to increase the US accuracy and reduce computed tomography and laparoscopy utilization.

With our algorithm of the right iliac and localized pain regions, ultrasound scanning optimizes the screening of patients with suspected acute appendicitis presented in the admission room.

Conflict of interest

The authors declare that they have no competing interests. There was no financial support or sponsorship.

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ORIGINAL ARTICLE

Antiviral Therapy

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Randomized Controlled Parallel-Design Clinical Study of the Efficacy and Safety of Intranasal Interferon gamma in Treatment of Influenza-Like Infections

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Abstract

Background: Influenza is a highly variable infection that can cause fatal complications. Universal approaches, such as general stimulation of the immune system to activate its natural antiviral capacities, seem to be a rational measure.

Methods: A total of 410 patients with influenza-like infections (ILI) were randomly assigned to one of three treatment groups and one control group. Interferon gamma (IFN-γ) was administered by intranasal introduction of 1 to 3 drops into each nostril 5 times per day daily for 5 days. The first dose of investigational medicine was given within 48h of the onset of the influenza-like symptoms. One drop of the solution contains 1,000 IU of active substance. All patients received basic complex therapy without any antiviral or immunomodulating agents. The patients were followed up for 7 days. Treatment efficacy was evaluated by the mean duration of symptoms (MDS), the period of viral antigen detection (VAD) measured after 1-2 and 4-5 days of treatment, and the incidence of complications. We used conventional indicators to evaluate the safety of IFN-γ in the treatment of ILI.

Results: The administration of 2 or 3 drops of IFN-γ in each nasal passage led to better outcomes manifested in the considerable (P<0.05) reduction of all acute respiratory symptoms, and therefore to a more rapid recovery. In these treatment groups, statistically significant decreases for MDS values, VAD period, and incidence of complications were registered. Intranasal IFN-γ in complex therapy of ILI was considered to be well tolerated and safe.(**International Journal of Biomedicine. 2018;8(4):327-322.**)

Key Words: Influenza • interferon gamma • respiratory tract infection • antiviral capacities

Abbreviations

ARTI, acute respiratory tract infections; **ILI**, influenza-like infections; **MDS**, the mean duration of symptoms; **VAD**, viral antigen detection.

Introduction

Interferon gamma (IFN-γ) is an antiviral and immunomodulating drug that prevents and treats ARTI caused by influenza or other viral respiratory infections. The IFN-γ activity against influenza virus infection, herpes simplex virus, HPV, and HIV, as well as against tuberculous and urogenital infections has been described in many studies. The mechanism of its antiviral action has been discovered, and antiviral properties have been shown in a range of preclinical and clinical studies.[1-6]

Influenza can be a very dangerous disease; it can lead to life-threating complications and death, especially in patients of risk groups.[7] The disease is caused by influenza viruses (the basic types are A, B, and C). Type A can cause serious complications and global pandemics. It has been detected in

the majority of clinical cases, and the subtype H3N2 generally dominates. Type B can also cause a rather severe infection; however, it never breaks out into a worldwide epidemic. Type C usually leads to mild acute respiratory tract disease.

According to the WHO reports, influenza takes the lives of up to 500,000 people around the world every year and potentially can kill millions of people. For instance, it affected half of the world's population in 1918 and caused the death of around 50 million people. Since the pandemic of 2009, type A influenza (mainly H1N1 and H3N2 subtypes) and type B influenza cause global co-infections every year with a high risk of dangerous complications.[8-10]

According to the Federal Service for Supervision of Consumer Rights Protection and Human Welfare, the mortality from influenza in 2017-2018 in Russia was around 70 people. Despite the reported decrease of mortality due to effective and widespread vaccination, which encompasses almost 60% of the country's population, the problem is not solved and the current results are not satisfying. From October to April, influenza still causes vast waves of affected patients, sick leaves, and hospital stays.[11]

The main difficulty is rapid mutations of the influenza virus. Every year, new strains appear and circulate in the population in different combinations. This significantly complicates the quick data collection necessary to make an effective vaccine; therefore, vaccination can rarely protect people.[12-14]

Another challenge is that alongside the influenza viruses, so-called «imitators» (the viruses of parainfluenza, adenoviruses, respiratory syncytial virus (RSV), coronavirus, etc.) could be a reason for acute respiratory tract disease. However, during the epidemic season more than 50% of upper respiratory tract infections are usually associated with influenza viruses.[15]

Today, influenza and other ARTI with similar clinical symptoms are commonly referred to as ILI. Since 2011, ILI has been defined as an acute respiratory illness with a measured temperature of 38°C or higher, and cough, with onset within the last 10 days. Although early clinical diagnosis of influenza is still a big challenge, this approach helps to detect influenza illness and distinguish it from other types of ARTI despite of non-specific and common symptoms, like fever. [10,16]

This article describes the results of a phase II, evidence-based clinical trial conducted to assess the efficacy of intranasal IFN-γ administered in several treatment schemes against ILI. The aim of the study was to evaluate the tolerability, safety, and efficacy of various treatment regimens of intranasal IFN-γ in complex therapy of adult patients with ILI.

The investigational drug has passed preclinical studies and phase I clinical trials. The study was performed in accordance with good clinical practice, with permission of the national regulatory authority, and under the approval of the Local Ethics Committee.

Materials and Methods

We conducted a randomized, controlled, parallel-design clinical trial. A total of 410 patients (mean age, 29.9-42.6 years) with ILI were examined prospectively, screened for inclusion, and recruited in one center if they satisfied the criteria (Table 1). The participants were enrolled during the period when influenza was announced to be present in the community (autumn-winter, 2005-2006). All participants provided the written informed consent.

Table 1.

Inclusion and exclusion criteria

No.	Inclusion criteria	Exclusion criteria
1	Men and women 18 to 60 y.o.	Pregnancy or lactation
2	Clinical symptoms of ARTI	Previous therapy with antitumor or immunomodulating drugs
3	Early stage of the disease (1-2 days)	Serious mental disorders that require treatment with antidepressants, major tranquilizers, hospitalization, or resulted in disability
4	Negative pregnancy test (for women)	Alcohol or drug abuse
5	Written informed consent	Diseases associated with immune system disorders (autoimmune diseases); diabetes; severe pulmonary, cardiovascular, renal pathology and other diseases that, from investigator's point of view, did not allow the patient to participate in the study
6		Inability or unwillingness to give the informed consent to participate in the study or to meet the requirements of the study

All the patients were randomly assigned to one of three treatment groups and one control group: with interferon gamma administered by intranasal introduction of 3 drops into each nostril (Main group I, n=110), of 2 drops into each nostril (Main group II, n=110), of 1 drop into each nostril (Main group III, n=110), and basic complex therapy without investigational drug (Control group, n=80).

The investigational drug—IFN-γ (Ingaron®, lyophilizate for the preparation of 100,000 IU solution for intranasal use, manufactured by PHARMACLON LLC, Russia, on the basis of patent No2214832)—was administered 5 times per day for 5 days. The first dose of investigational medicine was given within 48h of the onset of the influenza-like symptoms. One drop of the solution contains 1,000 IU of active substance.

All patients received basic complex therapy, including expectorants, antipyretics (in cases of temperature of ≥38.5°C), vitamins, and herbal inhalations, without any antiviral or immunomodulating agents. The patients were followed up for 7 days. The data on the concomitant therapy, including doses, frequency rates, course length, and names of drugs, were recorded.

Treatment efficacy was evaluated on the base of MDS, defined as the time from the first symptom onset to the last symptom release. Viral antigens were detected after 1-2 and 4-5 days of treatment. Incidence of complications was also assessed.

We used conventional indicators to evaluate the safety of IFN-γ in the treatment of ILI (vital signs, adverse reactions, and clinical laboratory tests). These parameters were compared

between the main groups and the control group. An adverse event was defined as any negative, unintended or unplanned effect (not only related to the investigational drug) on vital signs, symptoms, concomitant diseases, or laboratory parameters that changed after a patient had been enrolled in the study.

To evaluate efficacy and safety, subjective patient complaints were collected, and body temperature, blood pressure and pulse data were measured and recorded according to the study protocol. General blood and urine analyses were conducted before and after the treatment. All data were entered into individual case reports forms.

The etiology of the disease was established by immunofluorescence (IF) express-test with the identification of influenza, parainfluenza, RSV, adenovirus, and coronavirus infection. The IF analysis was repeated after 1-2 and 4-5 days of treatment. Specimens were collected from the upper respiratory tract (nasal passages) using swabs; blood and urine samples were prepared and tested in the local laboratory using routine methods.

Statistical analysis was performed using statistical software package SPSS version 17.0 (SPSS Inc, Chicago, IL). 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±standard deviation (SD) for continuous variables. A 95% confidence interval was calculated. For data with normal distribution, inter-group comparisons were performed using Student's t-test. A probability value of $P<0.05$ was considered statistically significant. Baseline data (four sets) included demographic indicators, and time from the first symptom onset before the intervention.

Results

Baseline demographic characteristics of patients and the kind of virus are shown in Table 2.

Table 2.

Baseline characteristics of 410 patients with influenza symptoms enrolled into the study

Baseline parameter	Main group I n=110	Main group II n=110	Main group III n=110	Control group n=80
Age	42.6±4.6	33.6±6.3	31.9±4.2	29.9±2.8
Female	37.8%	37.8%	53.6%	48.8%
Male	62.2%	62.2%	46.4%	51.2%
Smoking status	32.7%	19.1%	26.4%	23.7%
Time from the 1st symptom onset, h	34±6.9	38±7.2	35±9.3	37±6.3
Influenza A (H1N1)	15.5%	14.8%	17.6%	15.8%
Influenza A (H3N2)	17.2%	18.5%	15.7%	21.0%
Influenza B	10.3%	9.2%	2.0%	2.6%
RSV	15.5%	5.6%	17.6%	15.8%
Adenovirus	24.1%	31.5%	33.3%	28.9%
Parainfluenza I	19.0%	31.5%	29.4%	31.6%
Coronavirus	13.8%	3.7%	9.8%	10.5%

At the beginning of the study, no significant differences were observed among the groups regarding age, sex, smoking status, and time from the first symptom onset. Distribution of severity of fever and other ILI symptoms before treatment were also comparable among the groups (Figures 1 and 2). The mean duration of the illness before receiving the first dose was 36 hours.

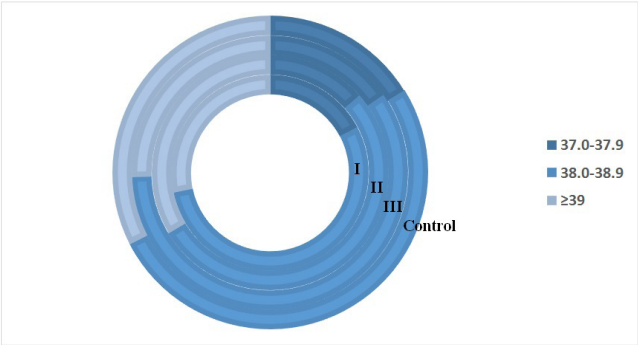

Fig. 1. *Distribution of fever among patients with ILI before treatment.*

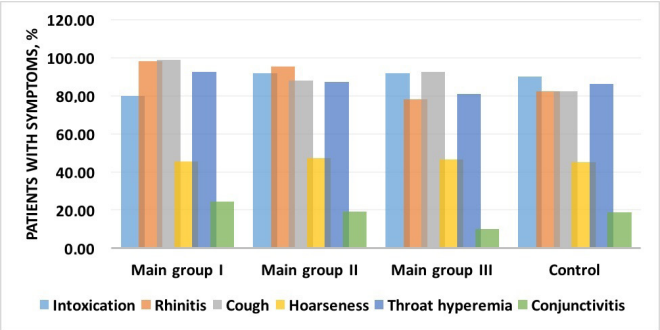

Fig. 2. *Distribution of ILI symptoms among patients before treatment.*

The obtained data showed that intranasal administration of IFN-γ in any treatment regimen at the first 2 days after the onset of the disease symptoms resulted in statistically significant reduced fever and rhinitis. High temperature fell crucially after the first day of treatment in Main groups I and II, and after the second day it was lowered by a statistically significant amount in Main group III too, as compared to the Control group (Figure 3).

However, the administration of 2 or 3 drops of IFN-γ in each nasal passage led to better outcomes manifested in the considerable ($P<0.05$) reduction of all acute respiratory symptoms (fever, intoxication and catarrhal symptoms), and therefore to a more rapid recovery (Table 3).

Thus, in these treatment groups, statistically significant decreases for MDS values were registered: 2 times for fever, and 1.7 times for general intoxication. The VAD period (according to the results of the IF examination performed at 1-2 days and 4-5 days after the start of treatment) was dramatically shorter in Main groups I and II, and demonstrated a significant ($P<0.05$) difference from the Control group after the first days of therapy (Figure 1).

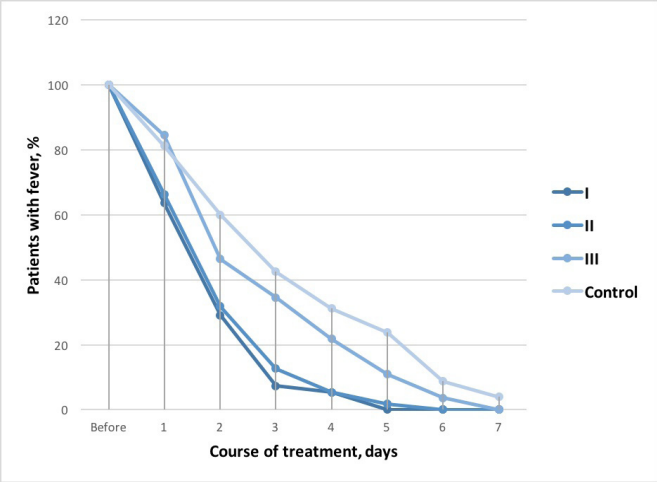

Fig. 3. *Dynamics of fever among patients with ILI during the treatment.*

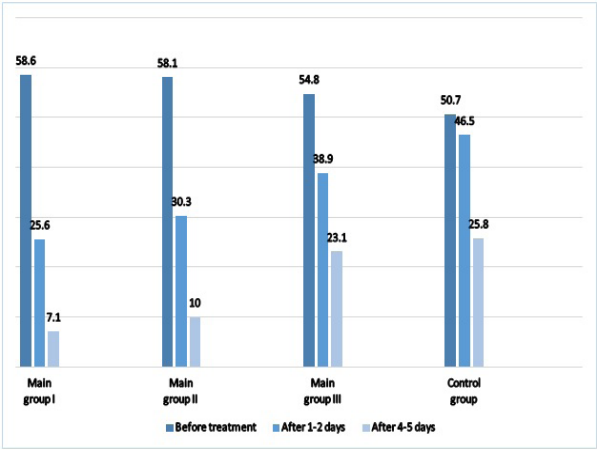

Fig. 4. *The duration of VAD in the nasal passages of patients with ILI.*

In general, the course of the disease was milder with half the number of complicated forms in Main groups I and II compared to the control patients (Table 4).

Table 4.

Frequency of complications in patients treated and not treated with IFN-γ

Group	Complications (%)	Including:				EI^
		bronchitis	pneumonia	sinusitis	tonsillitis	
Main I n=110	16.4	7.3	0	9.1	0	2.2*
Main II n=110	20.9	5.4	2.7	7.3	5.4	1.7*
Main III n=110	31.8	8.2	7.3	10.0	6.4	1.1
Control n=80	36.2	12.5	10.0	8.8	5.0	-

** -P<0.05 - in relation to the control group*

^Efficacy index (EI) was calculated as follows: the number of patients with complicated forms in control to the number of patients with complicated forms in the treatment group. It means how many times lower the rate of complicated forms of disease occurred in each therapeutic group in comparison with the control group.

No subjects reported either adverse reactions or individual intolerance. No pathological changes in hematological parameters were registered. Patients presented no subjective complaints about poor tolerability of the drug. No allergic reactions were reported. Therefore, intranasal IFN-γ in complex therapy of ILI was considered to be well tolerated and safe.

Discussion

IFN-γ, which is sometimes called immune interferon, is the most important proinflammatory cytokine. In the human body, it is produced by natural killer cells, CD4-Th1 cells and CD8 cytotoxic suppressor cells. IFN-γ receptors are present in macrophages, neutrophils, natural killer cells, and cytotoxic T-lymphocytes. IFN-γ activates effector functions of these cells, particularly, their antimicrobial activity, cytotoxicity, the production of cytokines, superoxide and nitrooxide radicals, and, therefore, destruction of intracellular pathogens.[1,4,17,18]

Table 3.

Results of efficacy evaluation of interferon gamma use to treat patients with ILI

Group	Number of patients	Duration of symptoms, days					
		Fever	Intoxication			Catarrhal symptoms	
			Total	Adynamia/ anxiety	Headache	Rhinitis	Cough
Main I	110	1.33±0.10*	2.18±0.16*	1.47±0.35*	1.00±0.15*	2.33±0.11*	3.11±0.78*
Main II	110	1.38±0.15*	2.16±0.18*	1.28±0.19*	1.07±0.19*	2.93±0.45*	3.85±0.19*
Main III	110	1.80±0.10*	3.71±0.34	2.93±0.15	1.67±0.59	3.03±0.21*	5.95±0.68
Control	80	3.22±0.29	3.78±0.26	3.21±0.20	1.64±0.33	4.01±0.82	6.33±0.70

**- P<0.05 - in relation to the control group.*

In 1995, Tomoda et al. suggested an important role of IFN-γ in the protection against influenza and other ARTI. At the first stage of the disease, endogenous IFN-γ ensures topical inhibition of viral replication (at the entry port of infection), removal of infected cells, and protection of non-infected cells due to *de novo* interferon synthesis. However, the activity of endogenous interferons is often insufficient to eliminate the infection. The administration of exogenous intranasal IFN-γ is justified for prophylaxis of infection in patients who have been exposed to an influenza virus, and for treatment of already infected patients, primarily in order to reduce the risk of dangerous complications.[19]

None of the currently available direct antiviral agents proved able to prevent severe complications of influenza. The amantadine group of medicines (amantadine, rimantadine) blocks the replication of type A viruses and is effective in 70%-90% of cases if administered as a prophylactic measure. At the same time, these agents have demonstrated up to 100% resistance since 2009. Additionally, the implementation of these drugs is limited because of side effects. On the other hand, neuraminidase inhibitors show their action when the infection has already attacked the organism and spread. However, the effect extends exclusively to influenza.[20]

IFN-γ acts not only as a preventive drug protecting cells against viral agents, but at the same time modulates immune response of the infected cells.

The preventive efficacy of IFN-γ in healthy adults has been demonstrated in a placebo-controlled clinical study, in which ILI incidence was reduced 2.2 times (P<0.05) during the administration period, and 1.9 times (P<0.05) within a one-month follow-up period after the administration, compared to the control group. On the base of this study, a positive budget impact for the health care system was shown with a subsequent 43% reduction in the estimated economic burden of influenza accompanied by a decrease in direct and indirect economic losses. Thus, the results of budget impact analysis confirmed the inclusion of Ingaron into complex influenza therapy as a cost-effective measure.[21]

Preclinical *in vitro* research into IFN-γ antiviral activity was conducted for the avian type of influenza (H5N2 subtype). Results showed a reduction in the cytopathic action of the avian influenza virus. Further studies are required to prove this data in humans.

It should be emphasized that the study described in the article recruited average-aged and healthy adults with no concomitant pathology. Our study was not aimed at investigating the effects of the drug on other groups of patients, including high-risk categories (children, pregnant women or elderly people). Further large-scale studies are required to confirm the obtained results in larger numbers of patients of all the ages and clinical conditions.

In conclusion, we have described the results of the clinical trial for treatment efficacy and safety evaluation of a range of daily doses of IFN-γ. The obtained data show that the inclusion of IFN-γ in the complex therapy of ILI in the early period of the disease (up to 48 hours) in adults can decrease the period of clinical symptoms (P<0.05), VAD (P<0.05), the incidence of complications (P<0.05) and, therefore, reduce the duration of the patient's stay on the sick-list.

Thus, IFN-γ can be recommended for the treatment of ILI in the dose of 2-3 drops into each nasal passage 5 times per day daily for 5 days due to its high antiviral and detoxification efficacy.

Conflict of interest

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

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Prevalence of Risk Factors of Thromboembolic Complications in Women after Major Joint Arthroplasty in the Republic of Sakha (Yakutia)

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Abstract

The aim of this study was to assess the risk factors for thromboembolic complications after total arthroplasty of large joints in women in Yakutia conditions to optimize the management tactics of this category of patients. The average age of women was 59.98±11.56 years in the age range from 50 to 70 years. In order to validate the study, women were divided into 2 groups. The main group consisted of 284 women undergoing total knee arthroplasty (Group 1). The comparison group included 147 women undergoing total hip arthoplasty (Group 2). The study demonstrated that hypertension was more common in patients of Group 1 than in patients of Group 2. However, the incidence of coronary heart disease and heart rhythm disorder was detected most frequently in patients with total hip arthroplasty. Obesity, thrombosis of the veins of the lower extremities, and liver disease were detected with almost the same frequency in women with total knee arthroplasty and those with total hip arthroplasty. The frequency of occurrence of complications depending on the risk factors for thromboembolic complications and the type of surgical treatment of the joint was equal in the two groups of studied patients. (**International Journal of Biomedicine. 2018;8(4):333-336.**)

Key Words: thromboembolic complications • women • total arthroplasty • large joints

Introduction

A significant prevalence of degenerative-dystrophic diseases of the joints of the lower extremities leads to a further search for effective methods of treatment.[1-3] Among the latter, arthroplasty of the joints occupies a rather significant place, which allows eliminating pain syndrome and restoring range of motion and supporting ability of the lower limb.[4,5] The risk of venous thromboembolic complications is maximal precisely in orthopedic practice, especially in endoprosthetic replacement of large joints. According to the summary statistics by W. Geertsetal (2004), after endoprosthetics of large joints in the absence of thromboembolic complications prevention, the incidence of deep vein thrombosis in the lower extremities reaches 40% to 80% and pulmonary embolism, 4% to 10%. The frequency of symptomatic thromboembolic complications in hip joint arthroplasty on the background of thromboprophylaxis is, according to various sources, from 1.3% to 3.4%, and in case of knee arthroplasty, from 1.7% to 2.8%.[6] Interestingly, fatal pulmonary embolism has remained consistent in primary total hip arthroplasty and total knee arthroplasty, between 0.1 and 2%, no matter which agents are used.[7,8] According to Pedersen et al.[9], a high level of comorbidity and general anaesthesia were strong risk factors for both venous thromboembolism and bleeding, with no difference between patients undergoing total hip and knee replacement. The assessment of the level of influence of various risk factors on the development of venous thromboembolic complications for many decades has been the subject of discussion in the medical literature, since an adequate prevention of venous thromboembolic complications is interconnected with the assessment of risk factors.[4,6,10] Considering the above-mentioned, the goal of our study was to

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assess the risk factors for thromboembolic complications after total arthroplasty of large joints in women in Yakutia conditions to optimize the management tactics of this category of patients.

Materials and Methods

This work is based on the results of a prospective clinical study of female patients who underwent knee and hip joint arthroplasty between 01.01.2012 and 12.31.2014. The average age of women treated in the trauma and orthopedic department was 59.98±11.56 years in the age range from 50 to 70 years. Large joint arthroplasty was performed in 592 female patients (Fig.1)

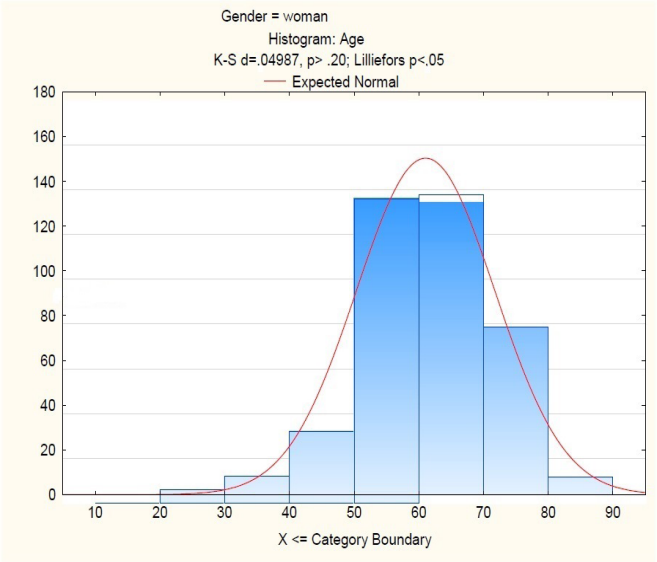

Fig.1. *The incidence of large joint arthroplasty depending on age*

Inclusion criteria: surgical treatment (total arthroplasty of the knee and hip joints) of degenerative-dystrophic diseases of the joint, false joint of the femur neck, systemic diseases, endoprosthesis instability, and written informed consent.

Exclusion criteria: lack of capacity to give informed consent; patient refusal.

The study was approved by the local Ethics Committee of M. K. Ammosov North-Eastern Federal University.

Patients eligible for all criteria included 431 people. In order to validate the study, women were divided into 2 groups. The main group consisted of 284 women undergoing total knee arthroplasty (Group 1). The comparison group included 147 women undergoing total hip arthoplasty (Group 2).

After the onset of hemostasis, all patients were prescribed prophylactic doses of anticoagulants in accordance with the clinical guidelines for the prevention of thromboembolic complications of the national standard of the Russian Federation. At discharge from the hospital, all patients were given recommendations for further prevention of thromboembolic complications; the goals of these drugs and possible complications were re-explained. It is also recommended to control the indicator of international normalised ratio to patients who took an indirect anticoagulant. Evaluation of the effectiveness of prophylactic treatment regimens for thromboembolic complications was carried out at the end of the course of prophylaxis and 12 months after surgical treatment.

Statistical analysis was performed using statistical software package SPSS version 19.0 (SPSS Inc, Chicago, IL). The normality of distribution of continuous variables was tested by one-sample Kolmogorov-Smirnov test. Quantitative parameters were presented as Median (Me) and 25th and 75th percentiles as Inter Quartile Range (IQR). Mann-Whitney U test was used to compare means of 2 groups of variables not normally distributed. The frequencies of categorical variables were compared using Pearson χ^2 or Fisher's exact test, when appropriate. A value of $P<0.05$ was considered significant.

Results and Discussion

Many studies in the literature describe a number of risk factors that act as factors for the development of venous thromboembolic complications. These include coronary heart disease, arterial hypertension, chronic circulatory failure, varicose veins of the lower extremities, obesity, and a history of thrombosis.[11-22] In systematic review performed by Zhang et al.[23], older age, female gender, higher body mass index and bilateral surgery were found to be venous thromboembolism risk factors for both total hip arthroplasty and total knee arthroplasty. Considering the above-mentioned, we carried out an analysis of these concomitant diseases in order to determine their significant effect on venous thromboembolic complications in the studied patients.

Analysis of the clinical characteristics revealed that in all patients the most frequently associated diseases were obesity, arterial hypertension, and coronary heart disease (Table 1).

Table 1.

The frequency of concomitant diseases, depending on the type of joint replacement

Concomitant pathology	Group 1 (n=284)	Group 2 (n=147)	P_{1-2}
Obesity	110 (38.7%)	68 (46.2%)	0.132
Varicose veins of the lower extremities	79 (27.8%)	39 (26.5%)	0.776
Hypertension	194 (68.3%)	82 (55.8%)	0.010
Coronary heart disease	75 (26.4%)	55 (37.4%)	0.018
Thrombosis of the veins of the lower extremities in history	2 (0.7%)	4 (2.7%)	0.090
Heart rhythm disorder	1 (0.35%)	8 (5.4%)	0.000
Hepatitis C. liver failure	7 (2.46%)	4 (2.7%)	0.872

Hypertension was more common among women in Group 1 (68.3%) than among those in Group 2 (55.8%). However, the incidence of coronary heart disease and heart rhythm disorder was detected most frequently in Group 2. Thrombosis of the veins of the lower extremities and liver disease were detected with almost the same frequency in women with knee prosthesis and those with hip prosthesis.

As can be seen from Table 2, the frequency of occurrence of complications depending on the risk factors for venous thromboembolic complications and the type of surgical treatment of the joint was equal in the two groups of studied patients.

Table. 2.

The frequency of complications depending on risk factors for thromboembolic complications and the type of joint replacement

Risk Factor	Complication* (n=14)		No complication (n=417)	
	Group 1	Group 2	Group 1	Group 2
Obesity	3	-	107	68
Varicose veins	3	2	76	37
Hypertension	3	2	147	80
Coronary heart disease	1	1	74	54
Thrombosis of the veins of the lower extremities in history	-	1	2	3
Heart rhythm disorder	1	-	6	8

**- no statistically significant differences between Group 1 and Group 2*

In conclusion:

The study demonstrated that hypertension was more common in patients with total knee arthroplasty than in patients with total hip arthroplasty. However, the incidence of coronary heart disease and heart rhythm disorder was detected most frequently in patients with total hip arthroplasty. Obesity, thrombosis of the veins of the lower extremities, and liver disease were detected with almost the same frequency in women with knee prosthesis and those with hip prosthesis. The frequency of occurrence of complications depending on the risk factors for venous thromboembolic complications and the type of surgical treatment of the joint was equal in the two groups of studied patients.

Conflict of interest

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

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Compensation of a Cavitary Bone Defect in Conditions of Implantation of Mesh Structures from Titanium Nickelide

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Abstract

The purpose of our research was to study reparative osteogenesis for implanting mesh structures of titanium nickelide into a cavitary bone defect.

Methods: The authors modeled cavitary defects of femoral metaphysis experimentally in Wistar rats divided into an experimental and a control group. The study duration was 60 days in total. Scanning electron microscope JSM-840 (JEOL, Japan) equipped with energy dispersive X-ray analyzer (INCA 200, Oxford Instruments) was used.

Results: Under implantation, the defect was filled with cancellous bone the volumetric density of which exceeded control values more than 1.5-fold (P<0.001). The implant had biocompatibility, osteoconductive and osteoinductive properties, and it stopped inflammatory processes. The membrane protective barrier, which prevented connective tissue sprouting, was formed on the surface of the implant in the defect periosteal zone. The osteointegrative junction was formed and persisted up to the end of the experiment. Reparative osteogenesis was performed by direct intramembranous and apposition type.

Conclusion: The implant of three-dimensional mesh titanium-nickelide structures has marked osteoplastic properties, and it can be successfully used in orthopedic surgery. (**International Journal of Biomedicine. 2018;8(4):337-341.**)

Key Words: reparative osteogenesis • titanium nickelide implant • cavitary bone defect • osteoplastic properties

Introduction

Connective tissue ingrowth from the periosteal surface is the main obstacle to restitution of large-volume bone defects that are caused by a higher rate of migrating fibroblasts compared to that of osteogenic cells.[1,2] This can inhibit the reparative osteogenesis process completely or partially, as well as be a cause of the defect filling with dense connective tissue of scar type. In order to create optimal conditions for formation of organotypic regenerated bone, the technique of guided reparative osteogenesis was developed using the membrane technology, which prevented connective tissue sprouting.[3,4] For this purpose, we used the membranes of synthetic and natural materials, which, however, are not osteointegrated; they can cause an inflammatory reaction and tissue swelling, in which case a repeat surgical intervention is required.[5,6]

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The membrane barrier over the bone defect that has good contact with the edges of the defect creates a closed space between the "mother" bone and the membrane. The membrane acts as a mechanical protective barrier, preventing the germination of connective tissue into the defect. Only the cells responsible for reparative osteogenesis fall into the defect from the surrounding bone tissue. In this case, there is a process of directed osteogenesis without the influence of other tissues. Numerous studies have been performed to elucidate the main features of directed bone regeneration,[8-11] but a number of issues remain unclear to this day. In particular, it is not clear whether there is a difference in the effect of regeneration from placement of osteoconductive or osteoinductive materials in the closed space between the bone and the membrane. Can individually manufactured membranes be used to achieve the desired shape and volume of bone tissue in the defect? New possibilities have arisen due to medical technologies that produce implants based on nickel and titanium, which are approached to bone tissue by their mechanical characteristics and are biocompatible.[12]

The purpose of our research was to study reparative

osteogenesis for implanting mesh structures of titanium nickelide (TN) into a cavitary bone defect.

Materials and Methods

Description of the intervention

Cavitary defects of femoral metaphysis were modeled in adult male Wistar rats in both the experimental and control groups (n=20 in each group); the defect volume was 0.02 cm^3, which accounted for about 40% of the total metaphysis volume. The volume of the metaphysis was determined by the volume of water displaced by it. The defect was filled with water by using a measuring syringe. All manipulations were carried out in accordance with "European Convention for the Protection of Animals used for Experimental and other Scientific Purposes" and approved by the National Ethics Committee. The implant was introduced into the defects in the animals of the experimental group; no additional manipulations were performed in the control group.

Characteristics of the implant

The implant had a mass of 0.017±0.001g, a volume of 0.02±0.001cm^3. The implant was a mesh framework made of nickelide-titanium thread (TH-10 brand of 90μm caliber) formed by the type of knitting with cells—through open pores of 100-300 μm diameter.[13] The thread was made of composite material comprising a core of nanostructured monolithic TN and a titanium-oxide microporous surface layer of 5-7μm (Research and Production Enterprise "MITs", Tomsk, Russia; Certificate No POOCRU.АЯ79Н18304). The micro relief of the surface layer of the filament was characterized by sharply pronounced roughness and nanostructuredness and the presence of a multitude of interconnected, open-capillary micro pores (Figures 1a, 1b, 1c). The porosity of the implant was 73.6±3.56%.

Methods of research and registration of outcomes

The animals were withdrawn from the experiment after 7, 14, 30 and 60 days (five animals were used for each time point). The bone meta-epiphyseal zone was sewn out, fixed in 2% paraformaldehyde and glutaraldehyde solution and embedded in araldite (without decalcification). The araldite blocks were studied using a scanning electron microscope JSM-840 (JEOL, Japan) equipped with energy dispersive X-ray analyzer (INCA 200, Oxford Instruments), and images (maps) were obtained in the characteristic X-ray emission of calcium atoms. The structures of bone tissue were visualized and the necessary measurements were made. The bulk density of bone tissue was determined (in %) as the ratio of the area occupied by bone structures in the image to the total area of the map. We then calculated the bone tissue compactness index (ratio of bone structure and non-mineralized components) and concentration of osteotropic macronutrients (calcium, phosphorus, magnesium, sodium and sulfur) in the bone regenerate.

Statistical analysis was performed using StatSoft Statistica v6.0. The mean and standard error of the mean were calculated. The Mann-Whitney (U Test) was used to compare the differences between the two independent groups. A probability value of $P<0.05$ was considered statistically significant.

Results

The process of bone formation that occurred from periosteum, endosteum, bone marrow and the damaged bone structures of the defect edges was observed in the both groups of animals 7 and 14 days after surgery. In the control group, a non-matured regenerated bone (of connective-tissue type) was formed in the periosteal zone; its collagen fibers grew from periosteum into the central zone of the defect as strip-like bundles.

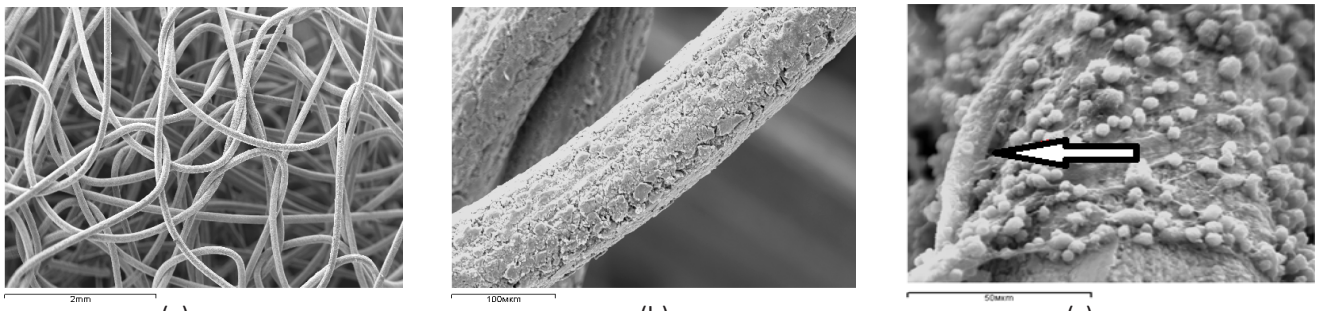

Fig. 1. *A mesh design made of TN implanted into the defect of the metaphysis of the femur: (a) - general view of the implant, (b) - micro relief of the surface layer of the implant filament, (c) - adhesion of slightly differentiated cells and newly formed capillary terminals (arrow) on the surface of the filament 7 days after the operation. Scanning electron microscopy (SEM), magnification: (a) - ×13, (b) - ×190, (c) - ×475.*

Table 1.

Bone tissue volumetric density in the metaphysis defect in the animals from the control group (Cont) and experimental group (Exp) and in the intact metaphysis of the contralateral limb

Parameters	Period of the experiment, days						Metaphysis of contralateral limb
	14		30		60		
Bone tissue, %	Cont	Exp	Cont	Exp	Cont	Exp	
	8.443±0.381	12.174±0.613[1]	13.543±0.681	19.764±0.962[1]	15.022±0.744	22.984±1.094[1]	25.072±1.212
Compactness index	0.092±0.005	0.143±0.012[3]	0.164±0.013	0.252±0.021[2]	0.181±0.013	0.310±0.021[2]	0.333±0.024

[1, 2, 3] Statistical significance of intergroup differences: [1]P<0.001, [2]P<0.01, [3]P<0.05

The defect was filled with loose connective and granulation tissue, where the foci of lymphocytic and plasmacytic infiltration and fibrin clots were located. The islets of reticulofibrous bone tissue, represented as a fine-cellular network of interwoven bone-osteoid trabeculae, were formed near the outer edges of the defect in the periosteum, as well as in the endosteum. Located near the defect's inner edges were randomly arranged fragments of the damaged bone structures, as well as separate unrelated foci of newly formed bone tissue as short bone-osteoid trabeculae lining the inner surface of the defect. In the control group, the bone tissue volumetric density in the defect and the compactness index of the regenerated bone amounted to about 30% (P<0.001) of the intact metaphysis values 14 days after surgery (Table 1).

In the experimental group, a thin membrane-like cover of connective tissue was observed around the implant threads and cells in the defect periosteal zone 7 and 14 days after surgery (Figure 2a). The cover formation began on the surface of the thread in the woven places and spread from the periphery of the cells to their center. The cover had a layered structure. The inner layer consisted of dense formalized connective tissue. Collagen fibers were collected into dense, circularly oriented, lace-like bundles, and they braided the implant threads in the form of a sleeve; they were firmly fixed to microporous surface of the threads, grew into the gaps between them and provided the fixation of the implant threads both between each other and in the bone defect (Figure 2b).

Areas of active appositional bone formation were observed below the connective-tissue cover in the endosteal and central zone of the defect and at its edges around the implant structures, as well as on their surface. A layer of reticulofibrous bone tissue of 300-400 μm thickness emerged on the surface of the implant thread directly forming an osteointegration connection (Figure 3a).

The implant threads in the osteointegration areas were coated with mineralizing bone matrix. Newly formed trabeculae grew into the implant's fine-cellular structure (Figures 3b and 3c). The results of quantitative studies (Table 1) evidenced significant activation of reparative osteogenesis, as well as of an increased degree of maturity of newly formed bone tissue in the regenerated bones of the animals in the experimental group compared to those in the control group. The bulk density of bone tissue in the defect in the animals of the experimental group was greater by 44.19%, and the compactness index by 55.56% compared to the values in the control group (P<0.001).

In the control group, the defect was filled with regenerated bone 30-60 days after surgery, where little-mineralized, dense, unformed, connective tissue growing into the defect from the periosteal surface prevailed. The initial stages revealed periosteal-intermediary uniting and forming a cortex with structure resembling cancellous bone (Figures 4a and 4b).

The operated metaphyseal zone acquired a marked conical shape. Little-calcified dense connective tissue prevailed in the periosteal zone of the regenerated bone. The fine-cellular bone structures of the periosteal regenerated bone fused with endostea newly formed trabeculae, and arcuate grew into the central zone of the defect and formed a thin crescent layer of newly formed cortical bone. Osteogenesis foci were observed in the central and marginal zones of the defect, where osteoid areas were revealed, as well as fragments of newly formed, little-mineralized, reticulofibrous bone trabeculae isolated from each other by wide interlayers of loose connective tissue with the cavities filled with lymphocytic and macrophage elements. The volumetric density of bone tissue in the defect, the index of compactness of the regenerated bone, and the content of calcium and phosphorus amounted to 50-60% of the intact metaphysis values (P<0.001) (Table 1, 2).

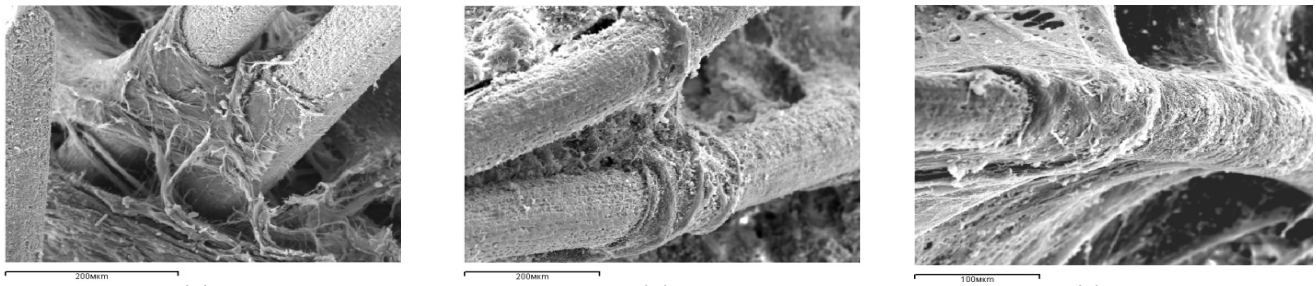

Fig. 2. *Connective-tissue cover on the implant surface in the defect 7 (a) and 14 (b, c) days after surgery. SEM, magnification ×160.*

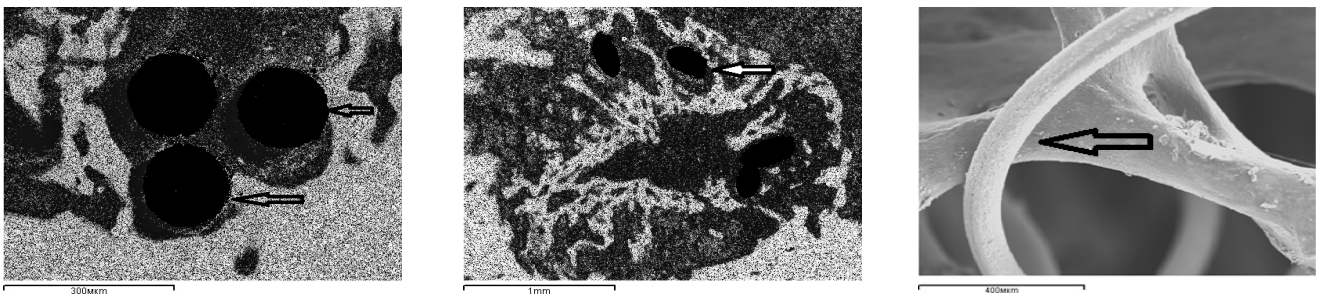

Fig. 3. *Reparative osteogenesis in the metaphyseal defect 7 (a) and 14 (b, c) days after surgery, arrows indicate the areas of osteointegration; a, b – maps of x-ray electron probe microanalysis, image in characteristic X-ray emission of calcium atoms, c – SEM (organic components removed with 6% sodium hypochlorite solution), magnification: a - ×100, b - × 25, c - ×70.*

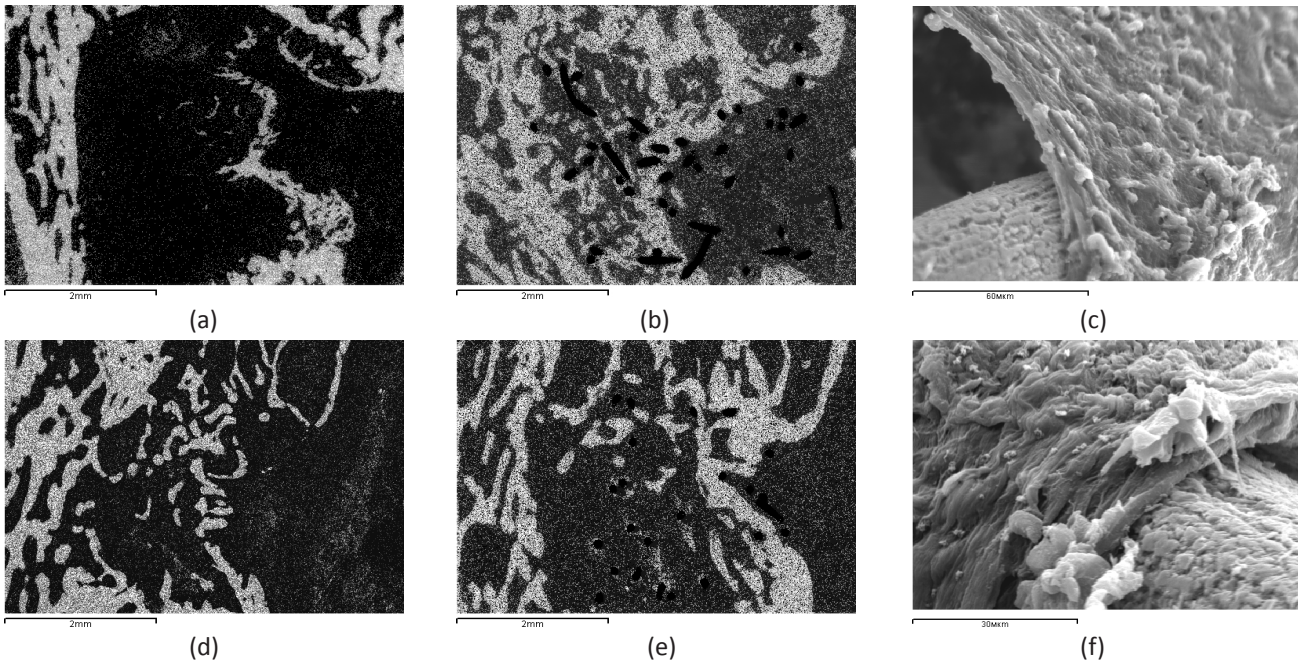

Fig. 4. *Reparative bone formation in the defect of femoral metaphysis in the control (a, d) and experimental (b, c, e, f) groups of animals; a, b, c - 30 days after operation; d, t, f - 60 days after operation; a, b, d, e – maps of x-ray electron probe microanalysis, mage in characteristic X-ray emission of calcium atoms, magnification ×20; c, f – scanning electron microscopy, magnification: c - ×670, f – ×800.*

In the experimental group, the area of bone defect was filled with regenerated bone, where cancellous bone prevailed 30-60 days after surgery (Figures 4b and 4e). A new area of cortical layer was formed, represented as compact bone of lamellar structure. Bundles of collagen fibers of the protective cover dense connective tissue were located on the periosteal surface of the defect around the implant, which formed an interweaving of the village fence type (Figures 4c and 4f). The implant threads were surrounded by osteoid or they were completely overgrown by newly formed bone tissue, thereby forming a composite—compact bone reinforced by TN. Bone tissue volumetric density, index of compactness, and degree of mineralization of the regenerated bone were somewhat less compared to the intact metaphysis values, but these differences were not statistically significant by the end of the experiment, and, at the same time, they exceeded the values in the control group by more than 1.5-fold (P<0.001) (Tables 1 and 2).

Table 2.

Content of osteotropic chemical elements in the regenerated bone of the control group (Cont) and experimental group (Exp) of animals 60 days after surgery and in the intact metaphysis of the contralateral limb (%)

Elements	Cont	Exp	Metaphysis of contralateral limb
Sodium	0.41±0.02	0.44±0.02	0.40±0.02
Magnesium	0.22±0.01	0.25±0.01	0.22±0.01
Phosphorus	1.93±0.04	2.94±0.13[1]	3.22±0.15
Sulfur	0.23±0.01	0.30±0.02[2]	0.22±0.01
Calcium	3.85±0.16	5.89±0.26[1]	6.44±0.31

Note: *[1, 2] Statistical significance of intergroup differences: [1]P<0.001, [2]P<0.05*

Discussion

Substitution or reconstruction of extensive bone cavity defects caused by congenital or acquired pathology is an actual medical and social problem. The main methods of surgical treatment are variants of bone grafting with the use of various synthetic, biological and composite materials. However, when studying the long-term results, it was found that most of these materials are not osseointegrated, but are surrounded by a fibrous capsule. The use of one's own bone (autotransplantation) is associated with an additional traumatic effect and is limited by the inability to take the necessary amount of autologous bone material, especially in children. In this case, there is also a risk of transmission of various diseases and the development of a number of serious complications of the immune nature, which are often accompanied by graft rejection and suppuration in the postoperative period.[14]

The results of this study showed that the implant we studied performed well the defect form, had good biocompatibility, and expressed osteoconductive properties. The microporous structure of the surface layer of the implant filaments ensured the adhesion of the cells of the regenerate and the formation of the osseointegrative compound, which was maintained until the end of the experiment. The development of osteogenic differentiation of cells on the surface of the implant was proved by the development of a specific calcified matrix. Regenerate tissues and blood vessels easily germinated into the implant without disrupting its integrity. In the periosteal region of the defect, a layer of dense connective tissue was formed on the surface of the implant, which served as a biological protective barrier preventing the germination of paraosal connective tissue. The defect was compensated by a spongy bone, the bulk density

of which at all stages of the experiment was more than one and a half times higher than the control indices, and its mineral composition approximated the parameters of the spongy bone of the intact metaphysis. Reparative bone formation was carried out by a type of direct intramembranous osteogenesis. In none of the cases were there signs of an inflammatory process, which confirmed the data we had received earlier.[15] For the first time during the implantation, an artificial composite biological tissue reinforced with TN filaments was obtained: dense fibrous connective tissue, spongy and compact bone. The fine-celled structure and micro porosity of the surface of the implanted structures created capillary properties, due to which endogenous bone morphogenetic proteins and growth factors were adsorbed. The functional activity of the latter—providing proliferation and accelerated differentiation of osteogenic cells; stimulating the synthesis of collagen, osteocalcin, and alkaline phosphatase; and activating the mineralization of the organic matrix of bone—ensured the osteoinductiveness of the implant.

Conclusion

The implant made of mesh structures of titanium nickelide is an effective osteoconductor and osteoinductor, and provides prolonged activation of reparative bone formation and spatial development of bone tissue in the defect. Atraumatism of surgical intervention and the absence of biological rejection reaction places the implant in the range of the most optimal osteoplastic materials, and its application seems theoretically grounded and promising, especially in patients with reduced osteogenetic and reparative potential, including in mature and elderly patients, as well as in children.

Conflict of interest

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Experimental Surgery

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Effect of p38 MAPK Inhibition on Apoptosis Marker Expression in the Process of Peritoneal Adhesion Formation

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Abstract

Background: Apoptosis, one of the most important mechanisms for maintaining homeostasis, is carried out under both physiological and pathological conditions. The aim of our study was to investigate the expression of markers of apoptosis through caspase-dependent and caspase-independent pathways during the reparative regeneration after serosal injuries of the peritoneum in the context of the prolonged p38 MAPK inhibition.

Methods: Peritoneal adhesions in the animal models were induced by a method developed by the authors that included opening the serous-muscular layer of the caecum with a 1cm cut followed by closing the wound with a Schmieden suture and scarifying a 1.5×1.5 cm area of the parietal peritoneum of the right lateral channel. Experiments were carried out on male 9-month-old Wistar rats. When closing the wound, control animals were intraperitoneally administered 3 mL of 0.9% sodium chloride solution (n=40) while experimental group rats were administered slow-release drug Seroguard® (Pharmasyntez JSC) (n=40). Animals were sacrificed within the period of 2 hours to 30 days post surgery. Expression of apoptosis markers was studied by immunohistochemical (Bcl-2, Bcl-xl) and immunofluorescent (PARP-1) staining.

Results: It is interesting that, in cases of the natural regeneration of the peritoneal injury, expression of anti-apoptosis markers at the injury site came in two waves: it was the most pronounced on days 1–3 post surgery while the second peak of activity was observed on day 14. Within this time window, granulation tissue was actively growing and mature connective-tissue vascularized adhesions were being formed. By the end of the observation period (day 30), expression of anti-apoptosis proteins at the injury site became extremely low and a significant reduction in the amount of connective tissue cells was observed. It was found that a prolonged inhibition of the p38 activity resulted in a moderate increase in Bcl-2 expression on days 3–7, and a decrease in the activity on day 14 was followed by another increase in expression by day 30. The Bcl-xl expression was observed 12 hours to 3 days post surgery and then it went down to the minimum. Positive PARP-1 staining observed on days 3 to 30, which reached its maximum on day 14, was also typical of the experimental group.

Conclusion: The performed study demonstrated that a prolonged p38 MAPK inhibition in the adhesion formation models results in the activation of fibroblast apoptosis at the reparation site, which, in the authors' opinion, predetermines a significant decrease in the adhesion formation in the experimental group.(**International Journal of Biomedicine. 2018;8(4):342-346.**)

Key Words: adhesions • peritoneal cavity • p38 MAPK • apoptosis • Seroguard • Bcl-2 • Bcl-xl • PARP-1

Abbreviations

AIF, apoptosis-inducing factor; **Bcl-2**, B-cell lymphoma 2; **Bcl-xl**, B-cell lymphoma-extra large; **MAPK**, mitogen-activated protein kinase; **PARP-1**, poly-[ADP-ribose]-polymerase-1.

Introduction

Apoptosis, one of the most important mechanisms for maintaining homeostasis, is carried out under both physiological and pathological conditions. Currently, the main mechanisms of apoptosis development in eukaryotic cells have been characterized and the research into apoptosis activators and regulators is underway. The interest in this issue lies in an opportunity to apply knowledge about the programmed cell death in medicine for the treatment of various diseases.[1]

The Bcl family of proteins play an important role in regulating apoptosis, and among them there are both pro-

(Bax, Bad, Bok, Bcl-xS, Bak, Bid, Bik, Bim, Krk, Mtd) and antiapoptotic types (Bcl-2, Bcl-xL, Bcl-w, Mcl-1, A-1, Boo).[2] It is assumed that a ratio of anti-apoptotic and pro-apoptotic proteins is crucial to actualization of the programmed cell death. The Bcl-2 family of proteins regulate apoptosis mainly at the mitochondrial signaling pathway[3,4] since signals of receptor-dependent signaling pathways generally bypass Bcl-2-mediated control. Bcl-2, Bcl-xL, and Bax proteins are capable of forming trans-membrane channels or being involved in their formation. For example, Bcl-2 directly or indirectly inhibits cytochrome C release from mitochondria [5] while Bax complexed with porin, on the contrary, forms a channel in the outer mitochondrial membrane for the release of cytochrome C and AIF into the cytoplasm. Apart from their channel-forming activity, proteins of the Bcl-2 family can act as adaptors that bind to proteins involved in apoptosis.

Since the Bcl-2 family of proteins is the key regulator of apoptosis, the abnormalities in its function have been implicated in many diseases, including cancer, neurodegenerative disorders, and autoimmune diseases.[6]

Systems that regulate DNA integrity are also important for apoptosis. PARP-1(cleaved p85) is known to participate in the repair of DNA damage. After DNA is cleaved by enzymes participating in the cell death (such as caspases), PARP-1 can deplete the ATP of a cell in an attempt to repair the damaged DNA. ATP depletion in a cell leads to the death of that cell. Moreover, PARP-1 has an ability to induce apoptosis directly via the production of poly(ADP-ribose), which stimulates mitochondria to release AIF. This mechanism appears to be caspase-independent.[7,8]

Apoptosis also plays a crucial part in the reparative regeneration processes. In particular, control over cellularity of the tissue being formed at the wound healing site is mediated by apoptosis.[9,10]

In the present research into the role that mitogen-activated protein kinase cascades play in regulating reparative regeneration after a peritoneal injury, the study of the relationship between the anti- and pro-apoptotic signal activity and the MAPK activity is certainly of interest.

Objective: to investigate the expression of markers of apoptosis through caspase-dependent and caspase-independent pathways during the reparative regeneration after serosal injuries of the peritoneum in the context of the prolonged p38 MAPK inhibition.

Materials and Methods

Peritoneal adhesion simulation

Peritoneal adhesions in the animal models were induced by a method developed by the authors that included opening the serous-muscular layer of the caecum with a 1cm cut followed by closing the wound with a Schmieden suture and scarifying a 1.5×1.5 cm area of the parietal peritoneum of the right lateral channel.[11-13] Experiments were carried out on male 9-month-old Wistar rats (n=80) weighing 220–250 g.

Animals were divided into 2 groups:

1) The control group (n=40) – intraperitoneal administration of 3mL of 0.9% sodium chloride solution warmed to 37° C when closing the wound (n=40);

2) The experimental group (n=40) – intraperitoneal administration of 3 mL of the slow-release drug Seroguard® (Pharmasyntez JSC), i.e., a sterile aqueous solution of conjugate of 4-(4-Fluorophenyl)-2-(4-methylsulfinylphenyl)-5-(4-pyridyl)-1H-imidazole[14] with polyvinyl imidazole, warmed to 37°C when closing the wound (n=40). Seroguard®'s main mechanism of action is a prolonged inhibition of the p38 MAPK activity in cells of the peritoneal surface layers.

The volume of fluid administered intraperitoneally was calculated in accordance with the data on the minimal volume needed to completely cover the peritoneum.[15] Animals were sacrificed within the period of 2 hours to 30 days post surgery (after 2, 6, 12, and 24 hours; on days 3, 7, 14, and 30).

Animals were housed in keeping with the rules for good laboratory practice (GLP). The experiments were performed in accordance with the norms for the humane treatment of animals, which are regulated by the International Guidelines of the Association for the Assessment and Accreditation of Laboratory Animal Care in accordance with the protocol approved by the Institutional Animal Care and Use Committee of the Irkutsk Scientific Center of Surgery and Traumatology.

Immunomorphological studies

Tissue material (intestinal suture area, adhesion attachment sites, peritoneum) was fixed in FineFix solution (Milestone, Italy). After fixation, tissues were processed, embedded in paraffin blocks, and cut into 5μm serial sections.

Then sections were deparaffinized. To study apoptosis marker expression, preparations were IHC stained with Bcl-2 primary antibodies (Abbiotec, Cat. N 250555, Lot 09110202) in a dilution of 1:300, Bcl-xl primary antibodies (Epitomics, Cat. N 1018-3, Lot E-07-12-01) in a dilution of 1:100, and Novolink Polymer System secondary antibodies (Novocastra, REF=7112, Lot 6006512) labeled with peroxidase. Sections were counterstained with 0.02% hematoxylin solution. Immunofluorescent staining[16] was carried out with the use of PARP-1 primary antibodies (Epitomics, Cat. N 1074-s, Lot CO11822) in a 1:50 dilution and Alexa Fluor 568 labeled secondary antibodies (Invitrogen, Cat. N A-11036 Lot 757102) in a 1:300 dilution. Tissue sections were visualized under a Nikon Eclipse 80i research microscope configured with DIH-M epi-fluorescence attachment.

Results

Expression of Bcl-2 family proteins during the reparative regeneration in animal models with serosal injury of the peritoneal cavity was studied both in the context of natural wound healing and under p38 MAPK inhibition by Seroguard® developed by the authors.

An increased expression of Bcl-2 and Bcl-xl was observed in control animals 2 hours after inducing adhesion formation. Single cells in the intestinal suture area and submucosal cells at the injury site were positively stained. No changes were observed 6 hours post surgery: Bcl-2 and Bcl-xl were expressed by single cells at the peritoneal injury site.

The intensity of staining for Bcl-2 marker expression

increased significantly by 12 hours post surgery while we also observed neutrophil infiltration around the suture, neutrophil infiltration and edema of the peritoneum, fibrin accumulation in the peritoneum and thickening of it, neutrophil and eosinophil infiltration of the submucosal intestinal layer, edema, and hemorrhages. Numerous cells at the peritoneal inflammation site were brightly stained for Bcl-2. Positive staining for Bcl-xl was also observed: Some cells in the peritoneal injury site were weakly stained while distinct staining was observed in the cytoplasm of cells at the intestinal suture area.

One day after surgery, the intensity of Bcl-2 and Bcl-xl staining was still growing in control animals against the backdrop of adhesion formation, pronounced neutrophil infiltration of the suture area and the submucosal and muscular layer of the intestinal wall, and pronounced neutrophil infiltration of the peritoneum. Peritoneal cells at the adhesion formation site were brightly stained for both markers, with many positively stained cells observed in the submucosal layer.

Three days post surgery, in the control group there were many cells positively stained for Bcl-2 found in the submucosal layer and at the adhesion formation site (Figure 1).

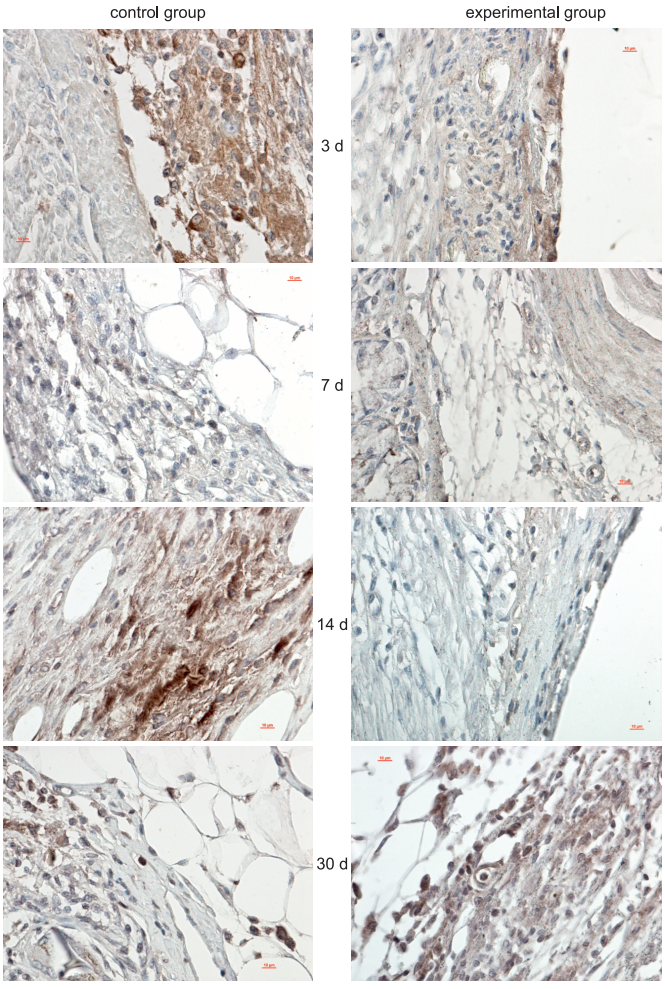

Fig.1.

Bcl-2 expression in control and experimental animals; immunohistochemistry with primary antibodies Bcl-2 (Abbiotec), 1:300, hematoxylin counterstaining.

At the same time, adhesions were being formed between the intestine and the omentum, between the site of the abdominal wall injury and the omentum, and between the abdominal wall and the intestine; and pronounced inflammation around the intestinal suture, widespread infiltration of the submucosal and muscular intestinal layer, evident signs of peritoneal inflammation, and growth of granulation tissue at the adhesion formation site (i.e., beginning of the fibroblastic phase of wound healing) were observed. Bcl-xl primary antibodies provided a very bright staining that was observed in the peritoneum inflammation area and the submucosal layer (Figure 2).

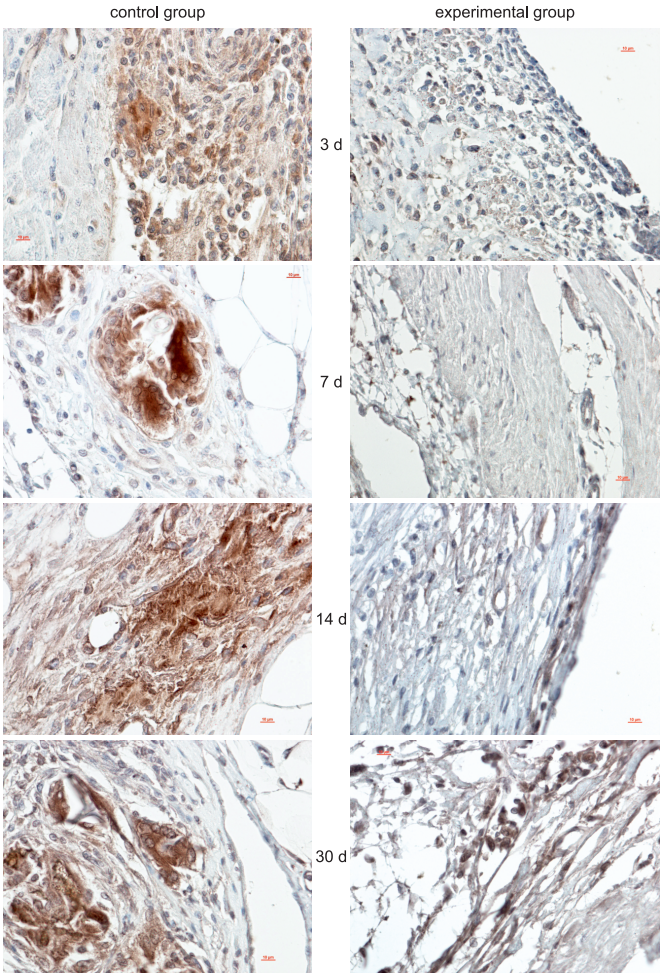

Fig.2.

Bcl-xl expression in control and experimental animals, immunohistochemistry with primary antibodies Bcl-xl (Epitomics), 1:100, hematoxylin counterstaining.

On day 7, rather bright Bcl-xl staining and weak Bcl-2 staining were observed against the backdrop of dense and long adhesion formation with no pronounced vascularization or presence of the capsule around the suture. What's more, the zones of staining for the two markers were different. In particular, an area of inflammation around the intestinal suture was positively stained for Bcl-2 while positive staining for Bcl-xl was observed in another area of the intestinal suture

inflammation that was different from the Bcl-2 positive one, and in the peritoneum, where it was bright.

On day 14, a very pronounced expression of Bcl-2 and Bcl-xl was still observed in submucosal cells, at the site of adhesion formation, and at the site of the anterior abdominal wall injury. Dense vascularized adhesions were being formed and connective tissue around the suture was growing. By day 30, in control animals Bcl-2 staining at the site of proliferation was minimal, and the same area was brightly stained for Bcl-xl against the backdrop of formation of multiple highly vascularized adhesions with a high density of collagen fibers.

The Bcl-2 and Bcl-xl expression in the experimental group differed from that observed in the control group. In particular, no positive staining for Bcl-2 and Bcl-xl was observed 2 hours after inducing adhesion formation. After 6 and 12 hours post surgery, we observed intestinal wall hemorrhages at the injury site and peritoneum deserosation with a moderately expressed inflammation of the area. Only single cells at the peritoneal injury site were positively stained for Bcl-2, with positive staining also observed around the suture while Bcl-x primary antibodies provided brighter staining of these areas.

After 24 hours, in the experimental group a moderate Bcl-2 staining was observed in the peritoneum at the adhesion site as well as around the intestinal suture with a moderate expression of Bcl-xl found in the same areas. Adhesions were formed between the intestine and the omentum, which was slightly attached to the suture site, and the peritoneum was moderately inflamed.

On day 3, the suture area and submucosal layer were brightly stained for Bcl-2 while Bcl-2 staining of the peritoneum was weak. All the three areas gave very weak Bcl-xl positive staining. There was minimal inflammation around the intestinal suture with a low cell packing density, mild inflammation at the peritoneal injury site, and the area of adherence between internal organs involved in the adhesion formation was minimal.

On day 7, a minimal Bcl-2 and Bcl-xl expression was observed. At the same time, short loose adhesions were being formed between either the intestinal suture and the omentum or the laparotomy wound and the omentum, and a mildly pronounced inflammation was observed.

On day 14, in the experimental group, no positive Bcl-2 staining was observed against the backdrop of sporadic adhesions in the form of a slight attachment of the intestinal suture or the laparotomy wound to the omentum and minimal inflammation of the intestinal suture. Cells at the suture area as well as in the peritoneum were weakly stained for Bcl-xl. On day 30, moderate staining for Bcl-2 was observed in the experimental group against the backdrop of single filmy unvascularized adhesions between the intestinal suture and the omentum with minimal contact and poorly developed connective tissue at the adhesion site. The suture area was brightly stained for Bcl-x while staining at the adhesion site was moderate (Figures 1 and 2).

To study expression of PARP-1(cleaved p85), we employed immunofluorescent staining. It was found that expression of PARP-1 could be observed in control animals only on day 7 after surgery, or later, whereas in the earlier periods of time there was no specific staining. On day 7, multiple PARP-1 positive cells were found in the submusocal layer with some single cells found at the peritoneal injury site (Figure 3).

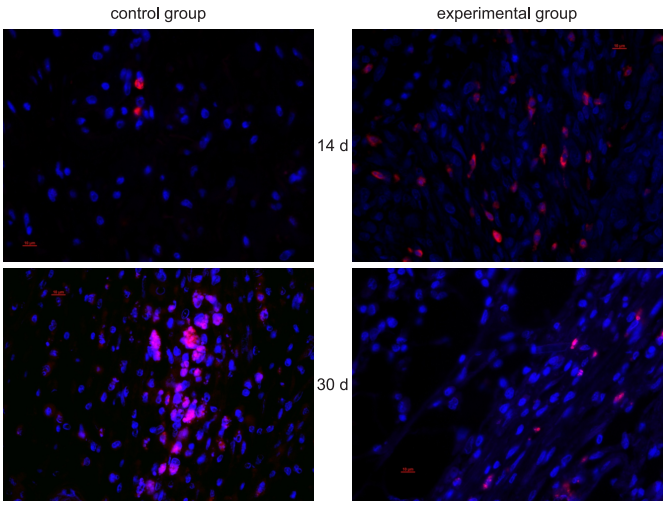

Fig.3.

PARP-1 expression; immunofluorescent staining; PARP-1 (cleaved p85) primary antibodies (Epitomics), 1:50, Alexa Fluor 568 labeled secondary antibodies, Dapi.

The number of positively stained cells at the site of adhesion formation increased, reaching its maximum by day 30.

In the experimental group, single positively stained cells were observed at the peritoneal injury site on days 3 and 7 after inducing adhesion formation. On day 14, we observed numerous PARP-1 positive cells at the suture area and some sporadic cells in the submucosal layer (Figure 3). On day 30, there were still a fairly large number of positively stained cells at the adhesion formation site, around the intestinal suture, and in the submucosal layer. Thus, the activation of apoptosis in animals of the experimental group was identified.

Discussion

Surgical treatment methods are widely used in current medical practice, and a big share of the surgical procedures is made up of approaches that suppose an invasion comes in the form of a major surgery, thus making it impossible to avoid damaging the serous membrane lining the cavity. So taking the above facts into consideration, we have performed some experimental studies aimed at clarifying pathogenesis of the serous lining reaction to an injury and evaluating a potential effect of p38 MAPK inhibition on the mechanisms of apoptosis.

Apoptosis is known to be one of the most important mechanisms for maintaining homeostasis. Moreover, its mechanisms are carried out under both physiological and pathological conditions.

The studies of the apoptotic activity during adhesion formation provide controversial data. Binnebösel et al. [17] carried out a prospective study of 40 patients with adhesive disease after abdominal surgery and found apoptosis activation in all cases.

At the same time, G.M. Saed et al.[18] studied cell lines derived from 5 patients with adhesive disease using polymerase chain reaction and found that fibroblasts from adhesions manifested sharply decreased levels of apoptosis marker expression compared to normal fibroblasts. In the authors' opinion, a decrease in the apoptosis activity could be indirectly responsible for the intensity of adhesion formation.

We have studied the expression of Bcl-2 family proteins during the reparative regeneration in animal models with serosal injury of the peritoneal cavity both in the context of natural regeneration process and under p38 MAPK inhibition.

We have found that in cases of a traumatic lesion of the peritoneum, pro-apoptotic and anti-apoptotic processes run simultaneously. While anti-apototic mechanisms prevail (more intense Bcl-2 staining) in the initial post-injury period, caspase-dependent pro-apoptotic phenomena take the lead at the later stages (more intense Bcl-xl staining). The PARP-1 activation indicates an increase in the frequency of cell DNA damage while the duration of that process induces the cell death via a caspase-independent pathway. Taken together, these processes result in eliminating a large number of cells, primarily fibroblastic ones, from the area of the aseptic inflammation at the peritoneal injury site where connective tissue is being formed.

It is interesting that, in cases of the natural regeneration of the peritoneal injury, expression of anti-apoptosis markers at the injury site came in two waves: it was the most pronounced on days 1–3 post surgery while the second peak of activity was observed on day 14. Within this time window, granulation tissue was actively growing and mature connective-tissue vascularized adhesions were being formed. By the end of the observation period (day 30), expression of anti-apoptosis proteins at the injury site became extremely low and a significant reduction in the amount of connective tissue cells was observed.[19]

It was found that a prolonged inhibition of the p38 activity resulted in a moderate increase in Bcl-2 expression on days 3–7, and a decrease in the activity on day 14 was followed by another increase in expression by day 30. The Bcl-xl expression was observed 12 hours to 3 days post surgery and then it went down to the minimum. Positive PARP-1 staining observed on days 3 to 30, which reached its maximum on day 14, was also typical of the experimental group.

The performed study demonstrated that a prolonged p38 MAPK inhibition in the adhesion formation models results in the activation of fibroblast apoptosis at the reparation site, which, in the authors' opinion, predetermines a significant decrease in the adhesion formation in the experimental group.

Conflict of interest

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Experimental Biology

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Electrophoretic Mobility of Red Blood Cells and Micronucleus Test in Exfoliated Buccal Cells as Stress Intensity Markers

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Abstract

The purpose of this study was to investigate the electrophoretic mobility of red blood cells (RBCs), cytomorphological and cytogenetical indices in exfoliated buccal epithelial cells of rats during the development of stress reaction.

Experiments were carried out on 60 white non-pedigree pubescent rats weighing 180-220 g. For stress modeling, the animals of experimental groups received a single intraperitoneal injection of epinephrine hydrochloride solution (0.1 mg/kg) or injections for 3 days of the experiment. Both the single and the repeated adrenalin injections provoked a decrease in the level of the RBC electrophoretic mobility. With a single adrenalin injection, the changes in RBC electrophoretic mobility are not so abrupt, with the subsequent development of the adaptive response and reparation of cytogenetic damage. With repeated adrenalin injections, the changes in RBC electrophoretic mobility are abrupt, and the number of pathological epithelial cells increases. (**International Journal of Biomedicine. 2018;8(4):347-350.**)

Key Words*:* red blood cells • electrophoretic mobility • buccal cells • stress

Introduction

Stress can be defined as a process in which environmental demands strain an organism's adaptive capacity, resulting in both psychological and biological changes that could place a person at risk for illness.[1] The relationship between stress and illness is complex and ambiguous. The critical factor associated with stress is its chronic effect over time. Research shows that almost every system in the body can be influenced by chronic stress.[2,3] Exposure of an organism to any of a variety of stressors markedly activates the sympathoadrenal and hypothalamic-pituitary-adrenocortical systems; and a "stress syndrome," according to concepts proposed by Langley, Cannon, and Selye, maintains homeostasis in emergencies such as "fight or flight" situations, but if the stress response is excessive or prolonged, any of a variety of clinical disorders can arise.[4,5]

There are many objective ways to measure human stress responses. Along with an evaluation of the activity of the hypothalamic-pituitary-adrenal axis biomarkers, the search for other methods, which could be easily used in any medical laboratory, is of current importance. Our study revealed that the electrophoretic mobility of red blood cells (RBCs) is an effective criterion of the severity of the organism's stress reaction to extreme effects.[6] Changes in the electrokinetic properties of RBCs can be considered as a response to the stress factor that is associated with consecutive activation of the basic humoral and endocrine systems. Thus, a decrease in the electrophoretic mobility of RBCs is determined by the activation of the sympathoadrenal system whereas its increase is determined by the activation of the pituitary-adrenal system.[7]

It has been shown that the stress influence is accompanied by a high concentration of glucocorticoids, which may disturb the secretion of pro-inflammatory interleukins (interleukin 1, 12, TNFα, interferon-γ).[8,9] In addition, it may provoke the

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apoptosis of activated T-and B-cells, and it drives excessive activation to the limitation of the immune system. Sometimes, it may drive the system to a pathological immunodepression.[10] The suppression of cellular immunity is accompanied by the appearance of cytogenetic abberant cells.[11] In this regard, it is very important not only to assess the degree of involvement of stress-realizing systems but also to define the genotoxicity of the reaction caused by the inclusion of these systems.

Currently, the micronucleus test (MT) is a non-invasive, simple and accessible method of assessing DNA damage.[11] It has been shown that MT is not less sensitive than the test of chromosome aberrations in bone marrow cells of animals, but it is less labour-intensive. The cytomorphologic research of buccal epithelium allows assessing the processes of epithelium proliferation and differentiation, the degree of inflammation, cell atypia, and nuclear polymorphism, as well as cytogenetic changes.[12]

The purpose of this study was to investigate the electrophoretic mobility of RBCs, cytomorphological and cytogenetical indices in exfoliated buccal epithelial cells of rats during the development of stress reaction.

Materials and Methods

Experiments were carried out on 60 white non-pedigree pubescent rats weighing 180-220 g. Animals were divided into 4 equal groups. For stress modeling, the animals of Group 1 received a single intraperitoneal injection of epinephrine hydrochloride solution (0.1 mg/kg), and animals of Group 2 received injections for 3 days of the experiment. The animals of control Group 3 received a single intraperitoneal injection of physiological saline solution, and those of Group 4 received injections for 3 days.

Blood samples were taken from the sublingual vein in 1 hour, 1 day, and 7 days after the epinephrine injection. Micronuclei (MN) identification in exfoliated buccal epithelial cells was performed 7 days after the epinephrine injection, considering the release of basal cells to the surface.[13,14]

The RBC electrophoretic mobility level was measured by the microelectrophoresis method.[15] RBCs were washed three times with 0.9% NaCl and collected by centrifugation. The cell suspension was made with 10mM tris hcl buffer (pH=7.4) and after that the RBC electrophoretic mobility was measured by registering the 100 mkm rbc transmission time in tris hcl buffer with pH of 7.4 and amperage of 12 мА. RBC electrophoretic mobility value was defined using the formula: U=S/T×H, where S – a distance to which the cells moved, T – time, H – a gradient of electric potential. The value of potential gradient was determined using the formula: H=I/g×χ, where I – amperage, g – chamber cross section, χ – electrical conductivity of the media.

The exfoliated buccal mucosal cells were scraped using a spatula, and cytosmears were stained with Giemsa. The specimen was analyzed with a microscope AXIOSTAR PLUS (Carl Zeiss, Germany), zoom 16×40 and 16×100. The number of cells with MN was counted. No more than 1000 cells were examined. The analyzed epithelial cells were well-expanded, not damaged, and without monolayer deposition. The cells having numerous microorganisms on the surface were excepted. MN were identified as round chromatinic bodies with a continuous smooth edge along with membrane and having the color of the same intensity as that of the main nucleus. Besides the binuclear cells, cells with anomalous nuclei, karyopyknosis, karyorhexis, anad karyolysis were also analyzed.[16]

Animals were housed in keeping with the rules for good laboratory practice. Experiment was performed in accordance with the Guide for the Care and Use of Laboratory Animals (the institute of Laboratory Animal Resources, 1996) and with approval of local Ethics Committee.

Statistical analysis was performed using the statistical software «Statistica». (v6.0, StatSoft, USA). The Shapiro-Wilk test was used in testing for normality. Baseline characteristics were summarized as frequencies and percentages for categorical variables and as mean±SEM for continuous variables. Student's unpaired t-test was used to compare two groups for data with normal distribution. A value of $P<0.05$ was considered statistically significant.

Results and Discussion

The obtained results are represented in Table 1. Both the single and the repeated adrenalin injections provoked a decrease in the level of RBC electrophoretic mobility. In Group 1, the maximal decrease in this parameter was found 1 hour after the beginning of the experiment, but it was restored by the seventh day of the observation. The repeated adrenalin injection provoked the maximal decrease of this index in the first day of the experiment. By the end of the first week, the index had increased by 40% in comparison with the first day of the experiment. The RBC electrophoretic mobility did not achieve the value of the control group.

Table 1.

Dynamic of RBC electrophoretic mobility index change (μm cm $B^{-1}c$) under different kind of influence

Group of animals	Period after the influence		
	60 min	1 day	1 week
Group 1	0.72±0.08*	0.83±0.09*	0.94±0.07
Group 3	1.02±0.02	1.07±0.03	1.00±0.03
Group 2	0.67±0.02*	0.54±0.03*	0.76±0.02*
Group 4	1.04±0.02	1.02±0.04	1.00±0.04

** - $P_{1-3}<0.05$, ^ - $P_{2-4}<0.05$*

The results of the RBC electrophoretic mobility study were identical to the findings that we received earlier when studying different kinds of stresses.[17] A literature review shows that the RBC electrophoretic mobility index decreases if there are some pathologies, such as chronic kidney disease, chronic brain ischemia, respiratory diseases, ischemic heart disease, or oncological diseases, as well as all kinds of intoxications. It was noted earlier that a decrease in RBC electrophoretic mobility

was associated with an increase in cell adrenoreactivity whereas the increase in RBC electrophoretic mobility was combined with an increase in cortisol concentration. Our study revealed that RBC electrophoretic mobility increased, after its primary decrease, more intensively in Group 2 than in Group 1. These data may be interpreted as a more considerable increase of endogenous cortisol concentration in the blood as a response to the repeated adrenalin injections. At the same time, it is well-known that the higher level of glucocorticoids provokes increasing the number of RBCs with chromosome aberrations.[17] This was discovered in the analysis of buccal epithelium in our study.

The cytogram analysis in the experimental groups showed that there were fewer normal cells and more cells with signs of cytological and chromosomal abnormalities, in comparison with the control groups.

The investigation of the animals of Group 1 revealed that the buccal cells had one or several micronuclei. The micronuclei were represented as oval or circular nuclear-like substances with smooth edges. We found a significant excess of MN in Group 1 (by 15%) compared to Group 3. Single cells with fragmented nuclei were observed. Their contours were without any spatial orientation. Cells with the initial stage of nuclear destruction, cells with chromatin condensation (corrugated nucleus with condensed chromatin) and cells with reniform vacuoles were frequently observed. The animals of the experimental groups had more cells with condensed chromatin (by 10%) and reniform vacuoles (by 43%) than animals of the control groups ($P<0.05$).

The revealed forms of cell necrosis make evident the destructive changes in the nuclear membrane and the disturbance of its barrier and transport functions. Karyolysis or nuclear dissolution represents an advanced stage of apoptosis and necrosis.[13,16] We did not find any statistically significant difference in the frequency of cells with karyolysis between the animals of the experimental and control groups. However, a significantly increased percentage of cells with initial signs of necrotic cell death in animals of the experimental group confirmed the development of stress reaction.

Karyopyknosis is a natural form of buccal cell apoptosis. The difference in the number of apoptotic cells in the experimental and control groups was not statistically significant. This proves that the natural mechanisms of buccal cell destruction remain safe.

Analysis of the cytograms in Group 2 showed that the number of cells with MN was greater by 25% than in Group 4. This difference means that there are pathological changes in buccal epithelium structure and a disturbance of the stability of genetic material. Epitheliocytes in animals of the experimental group had a decreased size due to a reduction in cytoplasm volume. We found many epitheliocytes with signs of the late stage of nuclear destruction (karyopyknosis, karyorhexis and full karyolysis) ($P<0.05$).

The analysis of cytological and nuclear disturbances in animals of the experimental groups showed that the cytogenetic disturbances (cells with micronuclei, cells with atypical nucleus) and cells with the initial stage of nuclear destruction (chromatin condensation) predominated in Group 1. Cells with signs of of karyopyknosis, karyorhexis and full karyolysis were identified to a greater extent in Group 2. The analysis of correlation between cytomorphological and cytogenetical indices of buccal epitheliocytes and RBC electrophoretic mobility revealed that the intensity of stress influence determinated the intensity of nuclei destruction. These effects are probably caused by the immunodepressive effect of highly intense stress. The compromised immune system can neither recognize nor eliminate the genetically disturbed epithelial cells; it contributes to the accumulation of disturbed cells and to the disturbance of cytogenetic stability.[11]

Conclusion

Our analysis shows that the adrenalin influence provokes at first a decrease in the RBC electrophoretic mobility index, and then an increase. With a single adrenalin injection, the changes in RBC electrophoretic mobility are not so abrupt, with the subsequent development of the adaptive response and reparation of cytogenetic damage. With repeated adrenalin injections, the changes in RBC electrophoretic mobility are abrupt, and the number of pathological epithelial cells increases.

Conflict of interest

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Clinical & Experimental Pharmacology

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Pharmacologically Active Tripeptide Leu-Ile-Lys in Indomethacin-Induced Gastric Ulcer

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Abstract

The aim of the study was to examine the effect of a new tripeptide, Leu-Ile-Lys, on an experimental indometacin-induced gastric ulcer in rats.

Materials and Methods: The experiment was performed with 24 male Wistar rats (average weight of 150 g). Rats were randomly divided into 3 groups: Group 1 (n=8) – the ulcer control group (IIGU), Group 2 (n=8) – the experimental group (IIGU+pre-treating with the tripeptide Leu-Ile-Lys), and Group 3 (n=8) – the comparison group (IIGU+pre-treating with omeprazole). The model of IIGU in rats was performed by a single intragastric administration of indomethacin (60 mg/kg in 1ml of physiological saline). In Group 1, indomethacin caused the appearance of severe injuries of the mucosa with the presence of extensive edema and leukocyte infiltration in the submucosal layer. In animals of Group 2, which were pre-treated with the tripeptide Leu-Ile-Lys, macroscopically gastric mucosa also looked smooth and atrophic changes were not found. Destructive changes were not severe; they appeared only in the form of small spot erosions. The number of spot erosions was 2.6 times less than in Group 1. The average erosion depth was 6.8 times less than in Group 1, and 2.0 times less than in Group 3.

Conclusion: Results of this study demonstrated the high, comparable to the action of omeprazole, gastroprotective activity of the new tripeptide Leu-Ile-Lys. (**International Journal of Biomedicine. 2018;8(4):351-354.**)

Key Words: peptic ulcer • Indometacine • oligopeptides • gastroprotective activity

Introduction

Peptic ulcer disease is responsible for substantial premature mortality worldwide.[1,2] The major causative factors of peptic ulcer disease are Helicobacter pylori infection and the use of nonsteroidal anti-inflammatory drugs and anti-thrombotic agents.[3] Despite the fact that the modern arsenal of antiulcerogenic drugs is quite extensive, a problem of finding new methods for the pharmacological treatment of gastric ulcer remains highly relevant, largely because existing drugs have limited efficacy and several serious side effects.

Previously, we discovered a gastroprotective effect of a peptide complex of pork kidney tissue that was manifested in a significant reduction of gastric mucosal damage in indomethacin-induced gastropathy in rats.[4] In the study of the amino acid composition of this peptide complex, it was found that it includes 15 amino acids, with the greatest mass percentage of leucine, isoleucine and lysine.[5] In this context, we suggest that these amino acids, and/or the peptides containing them, can be responsible for the gastroprotective effect. To test this hypothesis, we decided to synthesize the tripeptide Leu-Ile-Lys and examine its impact on the course of the experimental, indomethacin-induced gastric ulcer (IIGU), which was the purpose of the present study.

Materials and Methods

The experiment was performed with 24 male Wistar rats (average weight of 150 g). Rats were randomly divided into 3 groups:

Group 1 (n=8) – the ulcer control group (IIGU)

Group 2 (n=8) – the experimental group (IIGU+pre-treating with the tripeptide Leu-Ile-Lys)

Group 3 (n=8) – the comparison group (IIGU+pre-treating with omeprazole).

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Animals were housed in keeping with the rules for good laboratory practice. The experiments were performed in accordance with the norms for the humane treatment of animals, which are regulated by the International Guidelines of the Association for the Assessment and Accreditation of Laboratory Animal Care in accordance with the protocol approved by the Institutional Animal Care and Use Committee of Altai State Medical University.

The tripeptide Leu-Ile-Lys was synthesized at Shanghai Apeptide Co., Ltd. (Shanghai, China), supported by "Evalar" ZAO (Biysk, Russia). The purity of the sample was at least 98%.

In animals of Group 2, the tripeptide Leu-Ile-Lys was intragastrically administered in a dose of 11.5 mg/kg per day via gavage for 7 days prior to simulation of an indomethacin-induced gastric mucosal injury. The last tripeptide administration was performed 1 hour before the indometacine administration. The animals of Group 1 and Group 3 received physiological saline in equivolume amounts and omeprazole (37 mg/kg), respectively, by the same mode of administration. The model of IIGU in rats was performed by a single intragastric administration of indomethacin (60 mg/kg in 1ml of physiological saline).

The animals were euthanized 4 hours after the indomethacin administration. The stomachs were removed, cut along the lesser curvature, washed with a 0.9% solution of NaCl, and fixed in 10% neutral formalin. Gastric mucosal damage was scored by counting the total number of erosions, the number of linear deep erosions, the number of spot erosions, and the percentage distribution of various types of erosions. Paul's index was calculated for each type of injury by the formula: (N×K)/100, where N - average number of erosions on one animal, K - percentage of injured animals in the group.

For microscopic examination, specimens were stained with H&E to evaluate the tissue structure. Histochemical neutral mucopolysaccharides were detected using the Schick reaction, and acid mucopolysaccharides were detected by intensity of staining with Alcian blue (pH=2.5). The density of the inflammatory infiltrate in 1mm^2 was estimated using an Avtandilov`s ocular grid. Morphometric gastric mucosa were measured using the software Image Tool 3.0.

Statistical analysis was performed using StatSoft Statistica v6.0. Baseline characteristics were summarized as frequencies and percentages for categorical variables and as mean±SD for continuous variables. Multiple comparisons were performed with one-way ANOVA. The Mann-Whitney U Test was used to compare the differences between the two independent groups. A probability value of $P<0.05$ was considered statistically significant.

Results

The macroscopic examination of gastric mucosa surface of the Group 1 rats showed clearly visible changes in form of the linear and spot-shaped erosions (Fig.1). The results of microscopic examination in this group demonstrated that indomethacin caused the appearance of severe injuries of the mucosa with the presence of extensive edema and leukocyte infiltration in the submucosal layer. Gastric mucosa appeared atrophic, coating-patching epithelium was dystrophic. Necrotic changes in ulcerative defects reached the muscle layer. Massive deposits of hydrochloric acid hematin occurred in many erosions. Conditions of moderate edema and inflammation were fixed in the muscle layer.

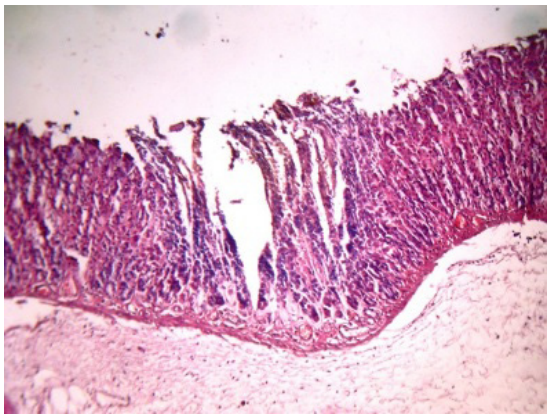

Fig. 1. *Deep linear erosion of gastric mucosa in a rat of Group 1. H&E staining, magnification ×100.*

In rats of Group 3, pre-treated with omeprazole, gastric mucosa macroscopically looked smooth and atrophic changes were not found. Weakly expressed destructive changes in the form of small spot erosions containing acid hematin were determined only in certain areas. All injuries were represented by spot erosions (Fig.2). Visible effects of mild inflammation occurred in the submucosal layer. The number of mucosal injuries was 6.9 times less than in Group 1 and the thickness of the mucous membrane was 1.8 times greater. The depth of spot erosions was 3.3 times less than in Group 1 (Table 1).

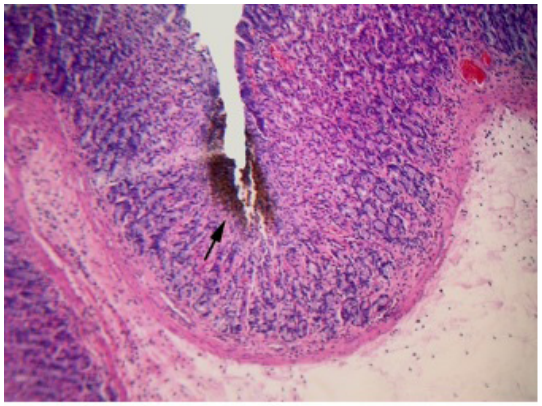

Fig. 2. *Spot erosion of gastric mucosa in a rat of Group 3. H&E staining, magnification ×100.*

Against this background, in animals of Group 2, which were pre-treated with the tripeptide Leu-Ile-Lys, macroscopically gastric mucosa also looked smooth and atrophic changes were not found. Destructive changes were not severe; they appeared only in the form of small spot erosions (Fig.3). Such destructive changes were observed in all animals.

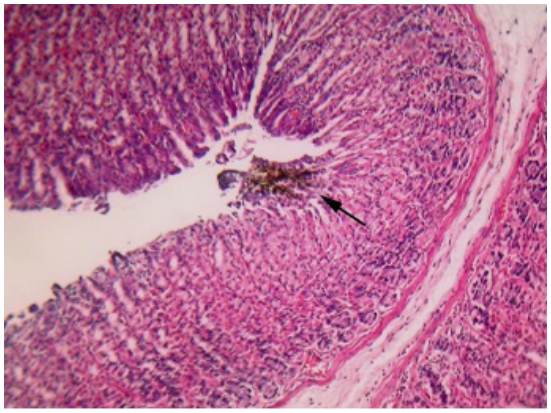

Fig. 3. *Spot erosion of gastric mucosa in a rat of Group 2. H&E staining, magnification ×100.*

Table 1.

Morphological indicators of the state of the gastric mucosa of rats in the studied groups

Index	Group 1	Group 2	Group 3	Statistics
The total number of injuries	9.6±1.7	1.75±0.25	1.4±0.4	F=165.764 P=0.000 P_{1-2}=0.000 P_{1-3}=0.0000
The number of linear erosions	4.8±0.9	0	0	
The number of linear erosion,%	51.1	0	0	
The number of spot erosions	4.6±1.4	1.75±0.35	1.4±0.4	F=32.972 P=0.000 P_{1-2}=0.000 P_{1-3}=0.000
The number of spot erosion,%	48.9	100	100	
Paul's index for the linear erosions	24	0	0	
Paul's index for spot erosions	16.8	1.75	1.4	
The thickness of the mucosa, microns	335.6±12.4	529.5±25.4	609.7±13.7	F=483.109 P=0.000 P_{1-2}=0.000 P_{1-3}=0.000 P_{2-3}=0.000
The depth of erosion, mm	385.75±23.7	56.7±6.4	115.8±5.2	F=1172.893 P=0.000 P_{1-2}=0.000 P_{1-3}=0.000 P_{2-3}=0.000
The density of the inflammatory infiltrate in 1 mm^2	1333.3±70.5	673.3±33.3	1040±211.7	F=51.563 P=0.000 P_{1-2}=0.000 P_{1-3}=0.001 P_{2-3}=0.04

The number of spot erosions was 2.6 times less than in Group 1 (Table 1). The average erosion depth was 6.8 times less than in Group 1, and 2.0 times less than in Group 3. Paul's index for spot erosions was 5.5 times less than in Group 1. The thickness of gastric mucosa on the periphery of erosions was 1.6 times greater than in Group 1. The density of the inflammatory infiltrate was 2.0 times less than in Group 1 and 1.5 times less than in Group 3. Parts of the columnar epithelium surface of mucosa, which were stained with Schick reagent, indicated a well-defined response to neutral mucopolysaccharides. In Stidmen staining of acidic mucopolysaccharides, an intensive staining was detected in cells of deep parts of the gastric pits. Weakly pronounced inflammation was observed in the submucosal layer.

Discussion

Thus, this study demonstrated a significant gastroprotective effect of the new tripeptide Leu-Ile-Lys on the model of indomethacin-induced gastric mucosal injury in rats. The effectiveness of the tripeptide Leu-Ile-Lys was comparable to the traditional drug, omeprazole, which is considered to be one of the most effective anti-ulcer agents.

It is commonly known that omeprazole suppresses basal acid secretion by inhibiting the proton pump in gastric parietal cells. At the same time, there were no data in the available literature showing that short-chain peptides may exhibit anti-secretory activity. It is possible that the mechanism of gastroprotective activity of tripeptide Leu-Ile-Lys, at least in part, can be determined by its ability to weaken the activity of the process of oxidation stress in gastric mucosa tissue, whose pathological role in the development of stomach ulcers is now recognized.[6,7] The reason to consider that suggestion is that the results of a previously conducted study showed us the pronounced antioxidant activity of the peptide complex from porcine kidney tissue in experimental urolithiasis and experimental gastropathy.[8,9] In addition, after the application of the tripeptide Leu-Ile-Lys, depth and density of the resulting erosion of the inflammatory infiltrate was significantly lower than after administration of omeprazole. Perhaps the tripeptide Leu-Ile-Lys has the ability to decrease an inflammatory reaction in the ulceration. Indirect confirmation of this ability can be regarded as recorded in our previous experiments that showed significant reduction of COX-2 expression in the gastric mucosa under the influence of the peptide complex from porcine kidney tissue in experimental gastropathy.[9]

In any case, the results of the present study clearly demonstrate the high gastroprotective activity, comparable to the action of omeprazole, of the new tripeptide Leu-Ile-Lys, patented by Altai State Medical University (priority date of 24.07.2018).

Conflict of interest

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Dental Technology

INTERNATIONAL JOURNAL OF BIOMEDICINE

The Influence of a Hemostatic Agent on Adhesion Strength and Microleakage of Composite Resin Restorations

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Abstract

The aim of the present study was to assess the side effect of an aluminum chloride hemostatic agent on adhesion strength and microleakage of composite resin restorations bonded with the one-bottle total-etch adhesive system.

Methods: We prepared 10 human tooth samples (extracted premolars for orthodontic purposes) in accordance with the Ultratest technique for the assessment of shear bond strength (SBS), and another 10 human tooth samples for microleakage assessment. The SBS tooth samples (n=10) were subjected to the two following tests: In Test 1, before traditional adhesive protocol, the aluminum chloride hemostatic agent was rubbed into a surface dentin for 60 sec with the help of a metal dento-infusor tip and washed with distilled water. In Test 2, just traditional wet bonding was performed. In the group of teeth (n=10) for microleakage assessment, we prepared two round artificial cavities of similar size (3 mm in diameter, 1 mm deep) in each tooth sample on the proximal surfaces with half in enamel and another half in root dentin. All created cavities (n=20) were divided into two subgroups. In cavities of Subgroup 1 (n=10), the adhesive protocol and filling with composite resin were performed after preliminary rubbing-in of the hemostatic agent. In Subgroup 2 (n=10), cavities were merely restored according to the rules for applying the one-bottle total-etch adhesive system. Assessment of microleakage was performed at the enamel margin and dentin margin. Scanning electron microscopy was used to evaluate the microstructure morphology of a hybrid layer, formed without the side effect of a hemostatic agent and after application of it.

Results: The average score of SBS was 7.42±3.5 kg in Test 2 and 3.87±2.45 kg in Test 1. Therefore, the side effect of the aluminum chloride hemostatic agent was detrimental to the bond strength of the composite resin to human dentin and significantly decreased the quality of adhesion by 1.92 times ($P<0.05$). The average scores of dye penetration through the enamel-composite microgap in both subgroups were low (0.5±0.52 CU in Subgroup 1 and 0.3±0.48 CU in Subgroup 2) and had no significant difference ($P>0.05$). However, the visual analysis of the dentin-composite junction of sectioned tooth samples demonstrated 2.1 times more microleakage in Subgroup 1 (1.7±0.95 CU) than in Subgroup 2 (0.8±0.79 CU), but the difference was not significant ($P>0.05$). In view of clinical situations with no possibility to escape the application of a hemostatic agent in cases of possible capillary hemorrhage and crevicular fluid leakage, it could be wise before running an adhesive protocol to cut off the portion of surface dentin that was exposed to an aluminum chloride hemostatic agent side effect. (**International Journal of Biomedicine. 2018;8(4):355-357.**)

Key Words: shear bond strength • microleakage • hemostatic agent • side effect

Introduction

The importance of composite adhesion to hard tooth tissues should not be underestimated. Cavities with close proximity to the gingiva may represent a great challenge to a clinician when placing a composite restoration. The chance of contamination of the operative field with blood and gingival crevicular fluid (GCF) is very high, even in the cases of cord gum retraction and rubber dam application.[1-4]

Aluminum chloride hemostatic agents were invented for dentists as materials of high efficacy and potency to prevent a conditioned tooth hard tissue from blood and GCF contamination. They serve restorative dentists to keep a prepared area dry and clean, which meets the needs of quality adhesive protocol.[5-8]

In accordance with the manufacturer's instructions, the hemostatic agent is for gum tissues and in some cases has to be intensively rubbed in the gums. Therefore, the effect on

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exposed dentin may be considered as a side effect of an agent or a proper contamination.

There are many studies indicating that such materials may induce changes in exposed dentin and impede the bond strength quality of a composite resin to dentin because of their acidic nature.[9-12] At the same time, bad composite adhesion can be the reason for low resistance to debonding stresses, which usually leads to extensive micro-leakage along the tooth-composite interface.[13]

Thus, the aim of the present study was to assess the side effect of an aluminum chloride hemostatic agent on adhesion strength (SBS) and microleakage of composite resin restorations bonded with the one-bottle total-etch adhesive system.

Materials and Methods

We prepared 10 human tooth samples (extracted premolars for orthodontic purposes) in accordance with the Ultratest technique for the assessment of shear bond strength (SBS), and another 10 human tooth samples for microleakage assessment.

The SBS tooth samples (n=10) were subjected to the two following tests: In Test 1, before traditional adhesive protocol, the aluminum chloride hemostatic agent (ViscoStat Clear, Ultradent) was rubbed into a surface dentin for 60 sec with the help of a metal dento-infusor tip and washed with distilled water. In Test 2, just traditional wet bonding was performed.

In the group of teeth (n=10) for microleakage assessment, we prepared two round artificial cavities of similar size (3 mm in diameter, 1mm deep) in each tooth sample on the proximal surfaces with half in enamel and another half in root dentin (Figure 1). All created cavities (n=20) were divided into two subgroups. In cavities of Subgroup 1 (n=10), the adhesive protocol and filling with composite resin were performed after preliminary rubbing-in of the hemostatic agent. In Subgroup 2 (n=10), cavities were merely restored according to the rules for applying the one-bottle total-etch adhesive system. Assessment of microleakage was performed at the enamel margin and dentin margin.

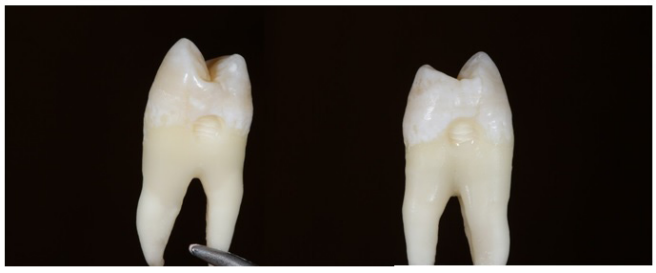


Fig. 1. *Round artificial cavities (3×1 mm) on tooth proximal surfaces.*

In both the SBS and microleakage parts of the study, the adhesive protocols were the same and the bonding agent was applied in accordance with the strict indications of the manufacturer's manual for the product. The etching gel was Fine Etch-37(Spider Dent, South Korea), the adhesive was Swiss TEC SL Bond, and the composite of choice was Palfique ESTELITE paste (Tokuyama, Japan). Light polymerization was performed with the help of Bluephase 20i (Ivoclar, Vivadent) in the "High" mode.

The SBS tooth samples were tested in the UltraTest machine at a crosshead speed of 1mm/min until adhesion failure occurred. Evaluations were registered in kilograms.

The microleakage tooth samples, after placement of restorations, finishing and polishing, were thermocycled, sealed with a sticky wax, and coated with two layers of nail varnish, with the exception of the restoration site and a 1 mm distance around of it. Tooth samples were stained in 2% methylene blue solution for 24 hours and sectioned through the centers of restorations.[14]

We evaluated dye penetration along the tooth-composite interface with a digital Canon EOS-5D camera mounted with Canon Macro Lens EF and scored on a nonparametric scale from 0 to 4.

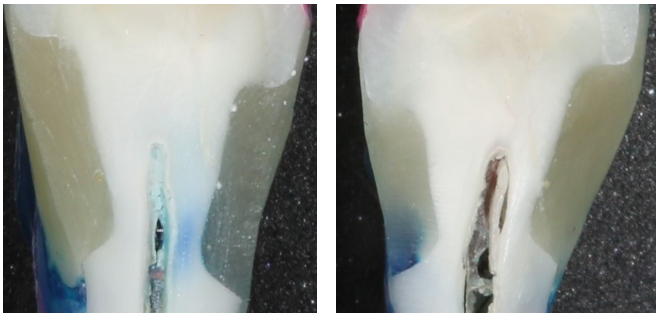
Randomly selected specimens, which were not subjected to microleakage evaluation, were scanned by a scanning electron microscope to evaluate the microstructure morphology of a hybrid layer, formed without the side effect of a hemostatic agent and after application of it.

Statistical analysis was performed using StatSoft Statistica v6.0. The mean (M) and standard deviation (SD) were calculated. The Mann-Whitney U Test was used to compare the differences between two groups. A probability value of $P<0.05$ was considered statistically significant.

Results and Discussion

The average score of SBS was 7.42±3.5 kg in Test 2 and 3.87±2.45 kg in Test 1. Therefore, the side effect of the aluminum chloride hemostatic agent was detrimental to the bond strength of the composite resin to human dentin and significantly decreased the quality of adhesion by 1.92 times ($P<0.05$).

As to the depth of microleakage (Fig.2), the average scores of dye penetration through the enamel-composite microgap in both subgroups were low (0.5±0.52 CU in Subgroup 1 and 0.3±0.48 CU in Subgroup 2) and had no significant difference ($P>0.05$). However, the visual analysis of the dentin-composite junction of sectioned tooth samples demonstrated 2.1 times more microleakage in Subgroup 1 (1.7±0.95 CU) than in Subgroup 2 (0.8±0.79 CU), but the difference was not significant ($P>0.05$).



(a) (b) (c) (d)

Fig. 2. *Macroscopic images of a sectioned tooth sample with methylene blue dye penetration along the tooth-composite interface: a,d – Subgroup 2; b,c – Subgroup 1.*

One of the basic requirements for performance of a quality composite resin adhesion is a clean tooth surface to be restored or bonded. That is why much attention is paid to isolation of the bonding area for prevention of contamination in time of placement of restoration.[15]

The application of aluminum chloride hemostatic gel for bleeding and gingival crevicular fluid control is a common method of tissue management, and such agents are named by many dentists as a «can't live without product».[1,2]

In vitro tests on adhesion strength and microleakage of resin composites substantiated with an analysis of SEM images are very informative and may have a prognostic value for dental practice. It has been shown that the side effect of an aluminum chloride hemostatic agent on the adhesion of a composite to human dentin is rather negative and should be considered as a tooth surface contamination.

Microscopic study of a hybrid interface discovered a decreased amount and length of adhesive tags formed in dentin after the exposure to the aluminum chloride hemostatic agent (Figures 3 and 4). The last observation additionally substantiates the fact of low bond strength of composite fillings and probable microleakage along the dentin-composite interface.

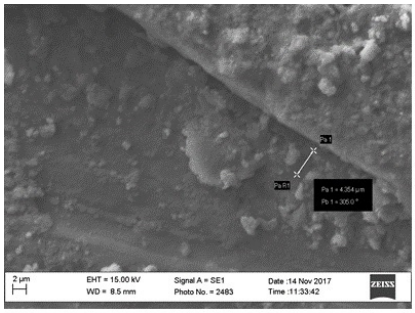


Fig. 3. *SEM image of dentin-composite interface after side effect of aluminum chloride hemostatic agent.*

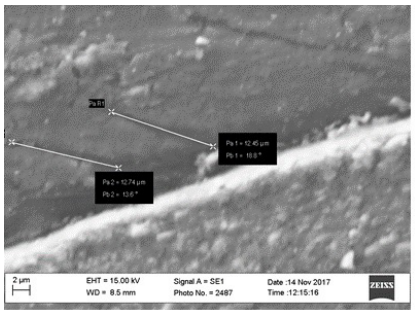


Fig. 4. *SEM image of dentin-composite interface without side effect of aluminum chloride hemostatic agent.*

Discrepancies in resulting significance of SBS and microleakage assessments may be due to the evident differences in character of debonding stresses used in tests. The nature of SBS examination is associated with the shear force application for registration of the level of the adhesion failure that occurred. As to the evaluation of microleakage, the detrimental effect of thermocycling may be compensated with close thermal expansion coefficients of tooth tissues and composite material.

Therefore**,** in view of clinical situations with no possibility to escape the application of a hemostatic agent in cases of possible capillary hemorrhage and crevicular fluid leakage, it could be wise before running an adhesive protocol to cut off the portion of surface dentin that was exposed to an aluminum chloride hemostatic agent side effect.

Conflict of interest

The authors declare that they have no competing interests.

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CASE REPORT

Dermatology and Venereology

INTERNATIONAL JOURNAL OF BIOMEDICINE

Pemphigus Erythematosus of the Scalp Developed Around the Postoperative Scar

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Moscow, the Russian Federation

Abstract

We present a clinical case reflecting the difficulties of differential diagnosis of pemphigus erythematosus (PE) in a 40-year-old male presented with erythematous topic lesions, pustules, and crusts on the skin of the scalp and face, which had existed for the previous 20 years of his life. In our case, clinical manifestations of dermatosis were atypical due to the presence of pustules and crusts impregnated with seropurulent exudates, resistant to antibiotic therapy. The patient had areas of atrophy on the scalp that could be a sign of discoid lupus erythematosus. The negative results of tests for LE cells and double-strand DNA antibodies, as well as ineffective treatment with plaquenil, antibiotics and itraconazole, helped to verify the diagnosis of PE. (**International Journal of Biomedicine. 2018;8(4):358-359.**)

Key Words: pemphigus erythematosus • differential diagnosis • biopsy • prednisolone

Introduction

Pemphigus vulgaris is an autoimmune skin disease, histologically characterized by akantholysis in epidermis.(1) Pemphigus is classified as a rare dermatosis; its frequency is 0.5-3.2 cases per 100,000 patients per year. Currently, there are four types of pemphigus: pemphigus vulgaris, pemphigus vegentans, pemphigus foliaceus, and pemphigus erythematosus(seborrheic) (PE).(2) It is important to mention that PE, also known as Senear-Usher syndrome, is the overlap-syndrome with features of lupus erythematosus (LE) and pemphigus vulgaris. The clinical signs of PE are various, but generally they are presented by small, flaccid bullae with scaling and crusting. Typically, PE lesions involve the skin of the scalp, face, upper part of the chest, and back.(3) It is necessary to remember that PE facial manifestations can be presented as a butterfly distribution on the bridge of the nose and on the malar areas, as seen in LE.(4) It is known that skin rash on the scalp typically has features of seborrheic dermatitis, but sometimes it manifests as circumscribed lesions with adherent massive crusts and weeping followed by alopecia and cicatricial atrophy.(4)

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Case Presentation

We present a clinical case reflecting the difficulties of PE differential diagnosis in a 40-year-old male presented with erythematous topic lesions, pustules, and crusts on the skin of the scalp and face, which had existed for the previous 20 years of his life. The patient considered these lesions related to a closed craniocerebral injury and subsequent neurosurgical operation with trepanation of the skull and removal of an intracranial hematoma in 1993. The skin rash first appeared at the periphery of the surgical scar. The patient applied topical antibacterial ointments without significant effect.

Observation of the skin surface of the scalp revealed massive dirty-yellow crusts impregnated with seropurulent exudate, solitary pustules, and big areas of erythema. Infiltrated pink-red papules and grey-white desquamation against the erythematous background were presented on the skin of the malar area (Figure 1).

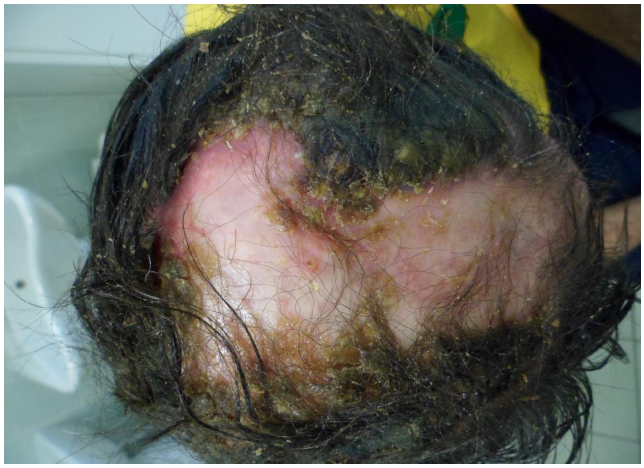

Fig. 1. *Clinical manifestations of pemphigus erythematosus.*

Patient received *ex juvantibus* therapy with plaquenil 200 mg 2 times per day for 2 weeks without significant effect. He was also administered subsequent antibiotic courses (doxycycline 100 mg 2 times per day for 10 days, azitromycin 250 mg 2 times per day for 10 days, and amoxicillin 500 mg per day for one week) and itraconazole 200 mg 2 times per day for 10 days without effect.

The skin biopsy revealed focal parakeratosis, neutrophilic infiltration of stratum corneum and the upper part of stratum spinosum, as well as a lack of stratum corneum at several areas and perivascular lymphohistiocytic infiltration with the presence of eosinophils in derma (Figure 2).

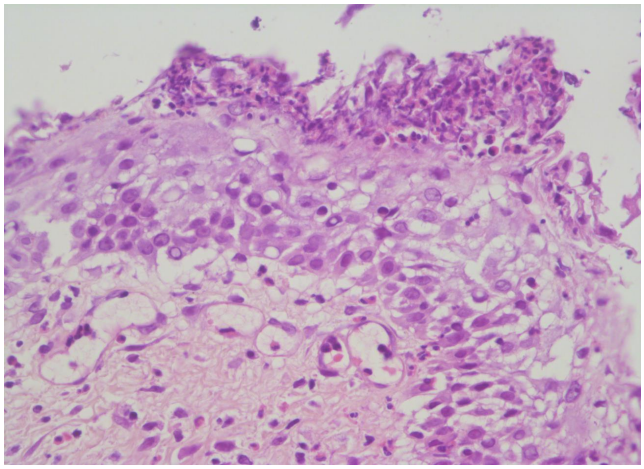

Fig. 2. *Histological examination of a patient with pemphigus erythematosus.*

According to the results of laboratory tests, LE cells and Demodex folliculorum were not found. The level of anti-dsDNA-NcX IgG in serum was 0.00ME/ml (N<100 ME/ml).

The culture test of the pustular content demonstrated a growth of *Staphylococcus aureus* (+++) and *Staphylococcus epidermidis* (+++). Due to technical difficulties, it was impossible to perform impression smear cytology to detect akantolythic cells.

According to the results of observation and laboratory tests, the patient was diagnosed with pemphigus erythematosus. He received prednisolone (starting dose 90 mg) with the clinical effect of the crust being gradually dried and rejected, and the number of pustules being reduced. After achieving clinical improvement, he was discharged with instructions to continue prednisolone 85mg with a gradual dose reduction as an outpatient.

Discussion

Diagnosis and verification of PE are difficult and require deep knowledge and experience. In our case, clinical manifestations of dermatosis were atypical due to the presence of pustules and crusts impregnated with seropurulent exudates, resistant to antibiotic therapy. It is worth mentioning that a similar case of the pustular form of PE was previously described by G.Yang et al. in 2014.[4]

The patient had areas of atrophy on the scalp that could be a sign of discoid lupus erythematosus. The negative results of tests for LE cells and double-strand DNA antibodies, as well as ineffective treatment with plaquenil, antibiotics and itraconazole, helped to verify the diagnosis of PE. However, it is important to remember that the biopsy is the most important diagnostic tool for bullous dermatoses.

Conflict of interest

The authors declare that they have no competing interests.

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