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REVIEW ARTICLE

INTERNATIONAL JOURNAL OF BIOMEDICINE

Biomedicine in the COVID Age: Opportunities, Responses, and Challenges

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Abstract

According to one of the earliest definitions, *biomedicine* means "clinical medicine based on the principles of physiology and biochemistry." Clinicians for quite some time preferred the use of the term *medical research* to describe what they considered the clinical findings pertaining to various issues related to clinical studies. Since the time when the basic molecules of life, deoxyribonucleic acids, were characterized and the genetic code elucidated, there has been great excitement, anticipation, and promise for the development of precision and personalized medicine. However, the progress has been considerably slow and at times disappointing. The unprecedented coronavirus disease created a worldwide panic and exposed all our weaknesses and unpreparedness. It also demonstrated a global demand for better public health infrastructure and preparedness to combat future pandemics. This unprecedented public health crisis acted as a great stimulus for putting together a concerted effort to develop vaccines. According to the experts, the time was right and within 48 hours after the information on the SARS-CoV-2 genome was posted, Moderna scientists had on paper a workable mRNA, which would code for the spike protein. The immune engineers at Moderna as well as BioNTech were able to put together a lipid nanoparticle delivery system for safe delivery of this precious cargo to the appropriate cells. Professor Cody Meissner at Tufts University School of Medicine in Boston says, "It is absolutely astonishing that this happened [COVID Vaccine development] in such a short time—to me, it is equivalent to putting a person on the Moon." It is indeed a great achievement, and it demonstrated the power of basic science and emerging technologies. The extraordinary success of mRNA vaccines has opened new avenues for mRNA-based therapies. mRNAs, siRNAs, and non-coding miRNAs will play a very important role as novel therapeutics soon. Furthermore, this success has acted as a catalyst for ongoing work on the use of small RNAs for therapeutic purposes. Having said that, I must say that there are a great many challenges that need to be addressed.**(International Journal of Biomedicine. 2021;11(3):241-249.)**

Key Words: SARS-CoV-2 • COVID-19 • biomedicine • vaccine

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Introduction

According to the Centers for Disease Control and Prevention (CDC), the 1918 influenza (H1N1) pandemic was the most severe pandemic in recent history. It is estimated that 500 million people or one-third of the global population were infected. The number of deaths was estimated to be at least 50 million worldwide. With no vaccines to protect from this virus and no antibiotics to treat the secondary bacterial infections, control efforts worldwide were limited to non-pharmacological interventions. As of this writing, global COVID-19 cases surpass 200 million with 4 million reported deaths. Compared to the 1918-1919 pandemic, we have done much better in terms of the number of deaths, which is less than 10% of what occurred with the H1N1 pandemic. We could have done much better, if only there was a global public health regulatory protocol in place, and the majority of individuals practiced public health best practices to prevent getting infected with this virus. The big difference in mortality also reflects on the advances made in biological sciences, immunology, biotechnology, and the development of a multidisciplinary approach to biomedical

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sciences. In this short review, we will briefly discuss the role of biomedicine, basic sciences, and emerging technologies in the improvements made in healthcare delivery. We will also discuss the difference between the top-down approach and the hypothesis-driven approach to problem-solving strategies. The unprecedented pandemic of SARS-CoV-2 has opened great opportunities for the development of novel therapeutics. Various stakeholders of healthcare delivery have responded in a great way during this pandemic. There are several challenges ahead for overcoming this pandemic as well as for developing strategies to face future pandemics.

SARS-CoV2, a sixteen trillion-dollar killer virus, has caused unprecedented health and economic crisis worldwide.[1] The novel coronavirus (2019-nCoV, SARS-CoV-2) epidemic first broke out in December of 2019 in Wuhan, China, and has been spreading worldwide like an uncontrolled forest fire.[2] Globally, 200 million individuals have been found to be COVID-positive with 4 million COVID-related deaths. The John's Hopkins COVID-19 tracker lists the following countries as the top five, in terms of COVID-related deaths: USA (607,865), Brazil (535,838), India (411,406), Russia (142,877), and France (111,597). According to the Chinese researchers, who were the first to describe this disease, the clinical manifestation of COVID-19 is heterogeneous.[3] The new variant delta seems to mimic common cold-like symptoms and thus presents an additional complication for early detection and quarantine of the infected individuals. The most prevalent comorbidity in China was hypertension (169%), followed by diabetes (8.2%). The new variant B.1.617.2, which was first identified in India, is found to be more transmissible than the earlier variants and is spreading fast in more than 75 countries.

Why is COVID-19 so controversial and at times so very confusing? According to an article in the Atlantic, "The confusion partly arises from the pandemic's scale and pace. Worldwide, at least 3.1 million people have been infected in less than four months. Economies have nose-dived. Societies have paused. In most people's living memory, no crisis has caused so much upheaval so broadly and so quickly. "We've never faced a pandemic like this before, so we don't know what is likely to happen or what would have happened," says Zoë McLaren, a health-policy professor at the University of Maryland at Baltimore County."

The rapidity by which this killer virus rampaged various countries caused an unprecedented healthcare crisis. No country was prepared for this kind of invasion of its people by an infectious agent. The suddenness of such a great wave of infection and deaths has had a devastating effect on existing healthcare delivery. This tsunami of COVID has exposed the unpreparedness of healthcare facilities. Suddenly the countries have realized deficiencies in healthcare infrastructure, human and material resources to handle such an unprecedented tsunami of COVID. There are not enough hospital beds, trained critical care workers, pulmonologists, doctors, nurses, ICU units, ventilators, medical supplies, medical-grade oxygen for therapeutic purposes, and general guidelines for public safety.

In an unprecedented effort, hundreds of scientists, clinicians, public health workers as well as laymen/women worldwide, are in a race against time, to answer myriad questions raised by individuals who are under panic, to develop better diagnostic tools, (preservatives, reactants, and characterization technology), novel drugs, interventions (pharmacological and nonpharmacological) and vaccines for SARS-CoV-2 virus.

According to the Coronavirus Disease 2019 (COVID-19)-Associated Hospitalization Surveillance Network (COVID-NET)(COVID-NET), which includes laboratory-confirmed cases in 99 counties in 14 states, the hospitalization rate increased with patient age. Those aged 65 years and older were admitted at a rate of 13.8%, with 50-64-year-olds at 7.4%, and 18-49-year-olds at 2.5 %. Hypertension was the most common morbidity among the oldest patients, with a prevalence of 72%, followed by coronary artery disease (50.8%) and obesity (41%). Is this the common pattern of comorbidity in all geographical areas? Not necessarily. The first largest study conducted in New York City concluded that pre-existing conditions such as hypertension and diabetes were highly prevalent, and the pattern was similar, to the data reported from China.[4] According to the WHO, in 2016 more than 1.9 billion adults, 18 years or older, were overweight. Of these over 650 million were obese. Over 340 million children and adolescents, aged 5-19, were overweight or obese. These obese individuals are at a higher risk for coronavirus disease. In the first meta-analysis of its kind, published on 26 August 2020, in Obesity Reviews, researchers pooled data from scores of articles capturing 399,000 patients. They found that people with obesity who contacted SARS-CoV-2 were 113% more likely than people of healthy weight to land in the hospital, 74% more likely to be admitted to an ICU, and 48% more likely to die.[5]

The interaction between the SARS-CoV-2 spike glycoprotein(S) and the ACE2, the cellular receptor for SARS-CoV-2, seems, to be the most critical point for the entry of the virus into the host cells. The high affinity of the S protein to the human ACE2 seems to facilitate the spread of this virus in human populations. This transmembrane spike (S) glycoprotein, forms homotrimers protruding from the viral surface and comprises two functional subunits, responsible for binding to the host cell receptor (S1subunit), and fusion of the viral and cellular membranes (S2 subunit). For all viruses of this group, the S unit is further cleaved by host proteases, at the S2 site of the fusion peptide. According to experts, four important enzymes are essential for the pathogenesis: the S-protein that facilitates virus entry through the ACE2 to the host cell surface receptor, the major protease of CoV3Clpro, the papain-like protease (PLpro) involved in the assembly of new viruses, and RNA-dependent polymerase (RdPr) that facilitate CoV RNA genome replication. The proprotein convertase family (PC) is composed of nine serine-secreting proteases and is widely involved in regulating various biological processes in normal and disease states. Therefore, the PC family, especially Furin can be considered the key player that mediates the maturation of S protein processing and recognition of membrane proteins.[6]

Interventions

In the absence of a cure, the only option people have to protect from this killer virus is to hide from it at any cost.

Public health experts worldwide advocated the use of facial covering, social distancing, better detection of the infected individuals (contact tracing), and strict quarantine of infected individuals. Those countries, which enforced such public health best practices, fared well, compared to those that did not. Researchers from the UK and the USA based on the data from 149 countries found that implementation of any physical distancing intervention was associated with an overall reduction in COVID-19 incidence of 13%. Data from 11 countries suggested overall effectiveness when school closures, workplace closures, and restrictions on mass gatherings were in place.[7] When various types of non-pharmacological interventions were compared, earlier implementation of lockdown was associated with a larger reduction in the COVID-19 incidence. According to the Global Dynamic Interventions Strategies for COVID-19 Collaborative Group, non-pharmacological interventions have been the mainstay for controlling the coronavirus pandemic.[8] This multicountry analysis demonstrated that intermittent reduction of infection rate (R) below 1 through a potential combination of suppression, interventions, and relaxation can be an effective strategy for the COVID-19 pandemic control. Unprecedented public health emergencies require unprecedented measures of public health best practices. One prime example of this approach is the Olympic Games in Japan to be conducted for the first time ever with no spectators. First Lady of the United States Dr. Jill Biden is visiting Tokyo, Japan, not as a spectator but to represent the U.S in the opening ceremony.

The unprecedented pandemic also gave tremendous opportunities for the drug discovery and development industries. Professor Cody Meissner at Tufts University School of Medicine in Boston says, "It is absolutely astonishing that this happened [COVID Vaccine development] in such a short time—to me, it is equivalent to putting a person on the Moon." "This is going to change vaccinology forever."[9]

How did this tremendous effort for drug discovery and development occur? This occurred just a few weeks after the first case was discovered in Wuhan, China, and its complete genome was characterized. Less than 24 hours after the genetic sequence was released by the Chinese investigators, researchers around the world started working on vaccine candidates. The response of the global *pharmaceutical* industries was as unprecedented as the pandemic itself. Different types of vaccines work in different ways to offer protection against the pathogen in question. The majority of the vaccines prompt our immune systems to recognize and protect the system from the virus that causes the dreaded disease. mRNA vaccines contain material from the virus that provides the instructions for making harmless spike (S) proteins that are unique for viral transmission. Protein subunits used in vaccines include harmless pieces of the viral protein that are an integral part of the virus instead of the entire germ. Vector vaccines contain a modified version of a different virus than one that causes COVID-19, but once the viral vector is inside the cells, the genetic material of COVID-19 gives cells the instructions to make a protein that is unique to the virus that causes COVID-19. Currently, there are more than 1,604 global, active, unique therapies in clinical trials; 389 antiviral trials, 299 anti-inflammatory drug trials, 125 monoclonal trials, and 104 vaccine trials.

Biomedicine in the COVID Age

In a general review like this, it is important to emphasize the role of basic sciences in the rapid development of therapeutics. The response of the pharma industry to the unprecedented COVID-19 pandemic provides some useful examples to illustrate this role. As early as January 10, 2020, Chinese researchers revealed the draft genome of the virus implicated in the Wuhan pneumonia outbreak. Jeremy Farra, head of the Wellcome Trust wrote, "Sharing of data good for public health, great for those who did the work. Just needs those incentives and trust." Scientists at Moderna, a biotech specializing in messenger RNA, were able to design a vaccine on paper in 48 hours, 11 days before the US even had its first recorded case, according to Antonio Regalado in the MIT Technology Review 124 (2), 2021. Within six weeks, Moderna had doses of vaccine ready for animal testing. The first hurdle they faced was to develop RNA molecules that can avoid the cytokine storm. They were able to do this by using chemically modified building blocks of RNA. Once they had perfected this technology, they had to engineer the delivery system for these molecules with ample protective coating.

In an editorial in Science Advances, Philip Yeagle uses this example as powerful testimony to the critical role of basic science in support of great scientific discoveries.[10] A key component of these vaccines is the carrier molecules that deliver the required mRNA into the cells, where it can read the information and make peptides for coding the viral spike proteins. That carrier is lipid nanoparticles, with their own unique history for targeted delivery of drugs. Successful use of lipid nanoparticles led to an explosion of ideas on targeted delivery of therapeutic molecules by encapsulating molecules of biological interest.

Even though the vaccine manufacturers now have a delivery system for synthetic mRNA to the cells, the use of nanoparticles of lipids for targeted delivery needs further exploration. If the drug packaged in the lipid nanoparticles are introduced into the blood, they tend to end up in the liver—a self-cleaning organ of the body. Weissman seems to have found a way to target the nanoparticles so that they wind up inside the bone marrow. He has not published this method, as it is a patentable technology. What this all means according to the experts is, that the fatty particles packaged with messenger RNA may become a way to edit genomes at massive scales and at a reasonable cost. Last spring, Moderna's CEO, Stephen Bancel approached the government to pay for the vast manufacturing centers to make messenger RNA. Later that month, as part of 'Operation Warp Speed', the US effort to produce vaccines, Moderna was effectively picked as the national champion to build such centers. The US Government gave Moderna $500 million to develop its vaccine and expand manufacturing. Despite the great success of vaccines against the COVID-19, they will not solve the problem associated with the pandemic. At the time of this writing, we already have new mutants (Delta variant) that are more transmissible and deadly, spreading worldwide. As the virus replicates, there

would be more mutations and more variants. Just recently, the Lambda variant of Peru has been reported from South America. Furthermore, there are already discussions about the need for a booster shot for all the vaccinated individuals. Public health workers and politicians have no clue whatsoever, as to how to convince a large population, who are reluctant to get vaccinated.

Novel Approaches for Potentiation of Vaccine-Induced Immune Responses

Helping B cells to produce antibodies is a major function of CD4+ T Cells. The role of T follicular helper (Tfh) cells in the modulation of immune function and in a range of diseases has been the subject of several investigations in the last decade.[11,12] Immunologists at St. Jude Children's Research Hospital have identified a biological pathway that modulates immune cells called Tfh cells, which mature into functional components of the immune system. These findings offer promise for developing drugs that activate the metabolic pathway to enhance the effectiveness of vaccines. Their studies revealed an endogenous pathway, called the CDP-ethanolamine pathway that selectively regulated Tfh cells.[13] To elucidate this endogenous signaling pathway, researchers performed CRISPR-Cas9 screening using pooled guide RNA (gRNA) library, that targeted associated genes. To discover a possible key-control pathway, Chi and his associates used genetic techniques to delete the T-cell multiple enzymes known to be key factors of such metabolic pathway. Then they introduced the deletion engineered T cells into mice and followed infection with a virus. These experiments demonstrated that a key metabolic pathway called the CDP-ethanolamine pathway, selectively regulated Tfh cells. Wang and associates studied the innate immune responses in 63 individuals who had recovered from COVID-19 and had received mRNA vaccines.[14] Authors concluded, "that immunity in convalescent individuals will be very long-lasting and that convalescent individuals who receive available mRNA vaccines will produce antibodies and memory B cells that should be protective against circulating SARS-CoV-2 variants."

Development of Biologics

Studies in large populations of COVID-positive individuals have demonstrated, that antibodies to the receptor-binding domain (RBD) of the viral spike protein appear, within few days of infection with the virus.[15] Follow-up studies with monoclonal antibodies (mAbs) against RBD, have been shown to decrease the viral load in patients with recently diagnosed mild/moderate COVID-19 infection. Regeneron reports positive data from the COVID-19 antibody cocktail trial. This is the experimental cocktail that was infused to Ex-President Mr. Donald Trump, when he was found to be COVID-19 positive. Regeneron is conducting phase 11 and phase 111 clinical trials. Regeneron Pharmaceuticals has developed a monoclonal antibody cocktail (Casirivimab and Imdevimab) that is supposed to reduce the risk of COVID-19 with a single injection. Eli Lilly also is developing a similar injectable preparation of antibodies to treat COVID-positive individuals. The US FDA granted emergency use authorization (EUA) in May to Sotrovimab, a super-antibody against COVID-19. Companies are designing next-generation antibodies modeled on those taken from COVID-19 positive individuals, whose immune systems can neutralize any COVID-19 variant and related coronaviruses too.[16]

National Institutes of Health is exploring nanobodies to combat COVID-19.[17] Authors claim that the nanobodies may be delivered via inhalation. For the first time, researchers at the University of Pittsburg School of Medicine, have tested monoclonal nanobodies, which are smaller in size, more stable, and cheaper to produce for inhalation treatment against coronavirus infections in a pre-clinical model. The nanobody research presents a great opportunity for therapeutic applications against airborne viral pathogens.

Development of Antiviral Drugs

In recent years, there is a continuous emergence of viruses with epidemic or pandemic potential. Recent examples include Ebola, Zika, Middle East Respiratory Syndrome (MERS-CoV), severe acute respiratory syndrome coronavirus 1 and 2 (SARS-CoV and SARS-CoV-2). The unexpected COVID-19 pandemic forced advanced nations to come up with an effective vaccine in short order. Having said that, I must indicate that vaccines are not the ultimate cure. Moreover, we do not have effective vaccines for many of the viruses mentioned above. Therefore, the development of broad-spectrum as well as specific antiviral drugs is essential. The U.S government spent more than $18 billion last year funding drug makers to develop a COVID vaccine. The new program announced on June 18, 2021, by the Department of Health and Human Services will invest $3 billion to advance the development of antiviral pills to treat COVID-19 as well as future virus outbreaks.

An antiviral drug must act at one of the five basic steps in the viral replications cycle: 1) attachment to the receptor and entry into the cells, 2) uncoating of the virus to release virions, 3) promote the synthesis of new viral components, 4) assembly of newly formed components into a new live virus, 5) release of the virus from the host cells. Nucleoside analogs represent the largest class of small molecule-based antivirals, which form the backbone of chemotherapy of chronic infections caused by HIV, hepatitis, and herpes virus. Nucleobase and nucleoside analogs (NNA) require extensive cellular metabolism to be converted to active metabolites. Currently, there are 41 NNA drugs approved by the US FDA of which 14 and 27 are used in cancer and antiviral therapies, respectively.[18-20] Researchers of St. Jude Children's Hospital, Memphis TN, USA, and National Child Center for Child Development, Tokyo, Japan, have demonstrated that NUDT15 polymorphism influences the metabolism and therapeutic effects of acyclovir and ganciclovir. These studies suggest that pre-emptive genotyping of these variants may be clinically important to mitigate toxicities of this class of drugs.

As discussed earlier, studies have revealed the integral role of proteases in SARS-CoV-2 viral spread and infection. Proteases belonging to the proprotein convertase family, including furin and furin-like serine proteases seem to play a

ubiquitous role in viral entry and spread.[21] Researchers have been investigating the NSP5 main protease as a potential drug target because of its involvement in processing the proteins coded from viral RNAs.[22] Acquired immunodeficiency syndrome (AIDS) of humans is caused by lentiviruses and various lifestyle behaviors led to a pandemic in the early 1980s.[23] AIDS the fatal disease which had no cure was spreading fast and to check its rapid growth and replication novel pharmacological approaches were developed. The interventional drugs tested belonged to five different categories with varied mechanisms of action.[24] They are 1) reverse transcriptase inhibitors, 2) protease inhibitors, 3) fusion inhibitors, 4) viral entry inhibitors, and 5) integrase strand transfer inhibitors. Despite the current emphasis on global vaccine development, such therapeutic virus management approaches will be researched and investigated for SARS-CoV-2 management also.

We already have discussed the role of Spike proteins (S) in the attachment of the virus to the ACE2 receptors through subunit S1 interaction. The host protein CatB/L transmembrane protease serine 2 (TMPRSS2) has been shown to be involved in the viral entry process. There is considerable interest in screening protease inhibitors targeting the viral or host factors involved in this essential process. The replicate/transcriptase complex (RTC) is composed of different enzymes and cofactors involved in post-translational polyprotein processing, RNA synthesis, maturation, and virus assembly and egress. Therefore, these steps seem to constitute ideal targets for novel drug discovery and development. Currently, there is a tremendous opportunity for drug repurposing for the management of virus infection, entry, replication, and overall management of the disease.[25] Another important area of interest is a rapid screening of antiviral drugs for repurposing in the COVID-age. Korean researchers developed a virtual screening assay for drug repurposing for COVID-19 by a screening of 6,218 drugs using a cell-based assay.[26] They developed an advanced virtual screening technique with pre-and post-docking pharmacophore filtering. They found seven compounds capable of inhibiting SARS-CoV-2 replication in Vero cells. Some of these promising inhibitory drugs included emodin, omipalisib/remedesivir, tipifamib/omipalisib, and tipifarnib/remedesivir.

Drug Discovery and Development in the COVID Age

The success of mRNA vaccines for COVID management has opened lots of opportunities for the therapeutic applications of synthetic mRNA. At the time of this writing, five people connected to the Moderna and BioNTech are now billionaires. In 2018, in the USA as well as in Europe, two RNA-based therapies have been approved for hereditary amyloidosis. Many RNA therapies are in the developmental stage and about a dozen are being tested in clinical trials. According to the experts, RNA therapies can be sorted into three categories: those that target nucleic acids, those that target proteins of importance, and those that encode proteins.[27] The major hurdle to RNA therapy has been delivering RNA to the correct target in the correct cells—simply put, targeted delivery. Intellia Therapeutics is testing a treatment protocol that packages CRISPR into RNA, and then into a nanoparticle for delivery to the liver for the treatment of inherited diseases such as sickle-cell disease and HIV. Since the lipid nanoparticles have been successfully used for packaging RNA for safe delivery to cells, it looks like a very simple and easy method. However, according to Professor Drew Weissman of Perelman School of Medicine at the University of Pennsylvania, it took him testing 40 different carriers before finding the ideal delivery vehicle—nanoparticles made from a mixture of fats.

Successful use of mRNA for therapeutic purposes has inspired a host of ideas about how to harness RNA for use in medicine. Rapid developments in this technology culminated in the 2018 approval, in both the USA and Europe, of two RNA-based therapies for the treatment of hereditary ATTR amyloidosis, a progressive and potentially fatal disorder in which abnormal proteins build up in nerves and in organs, such as the heart. The latest development in the use of this technology resulted in the completion of a trial between the biotech companies Intellia and Regeneron, in treating a rare disease with an IV infusion of the gene-editing technology CRISPR as the first delivery of the medicine to a human body. In this study, six people with a rare and fatal condition called transthyretin amyloidosis received a single treatment with gene-editing therapy. All experienced a drop in the level of a misshapen protein associated with the disease. Such emerging technologies are still in their infancy and are very expensive.[27] In late 2019, the US National Institutes of Health and Bill and Melinda Gates Foundation announced a $200 million grant for developing affordable gene therapies for use in sub-Saharan Africa. The target diseases were HIV and sickle-cell disease. How can such an emerging technology be developed at an affordable cost? Antonio Regalado writes in MIT Technology Reviews, Dr. Drew Weissman told me how they would make such cutting-edge treatments cheap and easy to use; the plan may depend on using gene-editing tools like CRISPR on a person's body, making permanent changes to the genome.[28]

Dr. Weissman says he intends to use this technology to try to cure sickle-cell disease by sending new instructions into the cells of the body's blood factory. He seems to be working in the monkey model, where T cells can be engineered to seek and destroy HIV and cure AIDS. Dr. Weissman thinks that RNA packaged in fatty acid nanoparticles may become a way to edit the genome on a massive scale, and on the cheap. According to experts, breakthrough CRISPR gene therapy could be a 'one and done' injection. CRISPR gene editing earned two of its discoverers the Noble Prize in 2020. A growing number of clinical trials are beginning to test gene therapies in humans. The therapy is made up of three parts. A tiny vesicle made up of lipid nanoparticles for delivery carries a payload of CRISPR machinery: a strand of guide RNA and a sequence of mRNA coding for the Cas9 protein. This approach, if successful, would be a one-time treatment, targeting the genes, to silence the defective mechanism permanently.

Challenges for Drug Discovery and Development

There are limitations to what vaccines can do. Furthermore, large populations of the world have no access to these vaccines. "None of us is safe until we all are safe" is a

common motto about COVID-19, and it is the idea behind the COVAX program to provide global access to vaccination. The member states of WHO have been divided into two groups. One is made up of 98 more affluent countries which are funding subsidized free vaccine supplies to 92 resource-poor countries. Germany is one of the COVAX program's biggest benefactors, providing almost $1.2 billion. During her recent visit to the USA German Chancellor, Angela Merkel requested US President Joe Biden to support the COVAX efforts. According to the World Health Organization, to provide vaccines to all the countries, COVAX needs 45 billion dollars. A high-level independent panel of the G20 Nations, which is currently meeting in Italy, has urged the launch of a 'global deal' to prevent catastrophic costs of future pandemics. New York Times, July 9th, 2021, reported that the board of the International Monetary Fund (IMF) on Friday, July 7, 2021, approved a plan to distribute $650 billion in reserve funds to help poorer nations with their vaccine rollouts and pandemic recovery efforts. The plan must be approved by the IMF's board of governors. We hope that the governors of the board of IMF approve this global relief effort.

Even if the funds are made available, there are not many more vaccine doses available, because the EU and the USA have already secured a large majority of them. Whereas, in a country like the USA, which has plenty of vaccines available, a large population is reluctant to get vaccinated. Dr. Nadav Davidovitch, the head of Israel's association of public health physicians, said he believes people have an obligation to get vaccinated, particularly given the evidence that vaccine not only prevents the worst outcome but also may reduce the spread of the virus. Despite such warnings, large populations are reluctant to get vaccinated. In view of this situation, we will not be able to eradicate the COVID-19 pandemic any time soon. In the USA, Pfizer one of the vaccine developers announced that they are making booster shots. US Food and Drug Administration and the CDC say that there is no need for booster shots currently. Israel on the other hand is recommending booster shots, considering the dangers posed by the delta variant. The COVID-19 pandemic may remain with us for some time to come, and chances are eventually people may get herd immunity over a period. But till that happens, we will see a lot of suffering, economic loss, and mounting, preventable deaths worldwide. Even in an advanced country like the USA, where vaccination is free and is available for everyone, only about 47% of the eligible population is vaccinated with the two doses. Already Pfizer maker of one of the mRNA vaccines is planning a booster shot to combat the delta variant. Despite the protection offered by the modern vaccines against COVID-19, we cannot totally rely on the vaccines to eradicate this virus.

Biomedical Research and Healthcare

In an earlier article titled "Biomedical Research and Healthcare" in this journal, I discussed biomedical research innovations.[29] I also discussed President Barack Obama's billion-dollar precision medicine initiative. In the 1950s, Francis Crick and James Watson together at the University of Cambridge, England explored the structure of proteins. In 1962, these pioneer scientists were awarded the Noble Prize in Medicine for their work in determining the structure of DNA—the genetic code. The Human Genome Project, which began in October of 1990 and was completed in April of 2003, has provided the ability for the first time, to read nature's complete genetic blueprint for building a human being. National Human Genome Research Institute (HGRI) of the National Institutes of Health (NIH) has an extensive database on a variety of topics related to human diseases. From the time HGRI was initiated in the 90s, there is great expectation and excitement, about its possible contributions to improvements in healthcare. It is important to recognize the difference between genetics and genomics. Genetics is the study of single genes and their effects whereas genomics is the study not of single genes, but of the functions and interactions of all the genes in the genome. Despite the extensive database that HRG has created on a variety of 'omics' data, we are of the opinion that a well-thought-out hypothesis-based investigation will yield more valuable information than a 'Top down' approach like a precision medicine initiative. We also have advocated in our earlier articles, that treatment of the disease itself is better than focusing on the management of risk factors for a disease or cluster of diseases.[29-33]

In the following, few paragraphs we will try to provide some examples in support of our views on this topic. Let us consider coronavirus disease as an example as we have discussed this extensively in the last few months.[34-43] In News Feature in Nature July 8, 2021, Ewen Callaway writes, "Genome studies have discovered some genetic risk factors for disease — and could point to treatments."[44]

Since last March when SARS-CoV-2 became a pandemic worldwide, researchers around the world have scouted the genomes of more than 100,000 people with COVID-19, hoping to find genetic clues to who will be hit hardest by an infection with the virus SARS-CoV-2. According to Callaway, what has emerged from this global effort is a dozen or so genetic variants that have a strong statistical association with a person's chances of developing COVID-19 and becoming gravely ill with the disease.[44] Alessandra Renieri, a geneticist at the University of Siena, Italy, and an early member of the HGI (COVID-19 Host Genetics Initiative), says that each new genetic finding is like a piece of a puzzle. "Several pieces are coming together. I'm sure that the picture will be much more clear in the very near future." Having said that, I must emphasize the importance of gene expression in immune modulation. From a different perspective, if we analyze the same cohort with a different parameter for severity, then it becomes evident that the severity of the coronavirus disease was highly correlated with the presence or absence of underlying risk factors such as hypertension, excess weight, obesity, diabetes, and vascular diseases.[34-43]

Obesity is a recognized risk factor for severe COVID-19, possibly related to chronic inflammation that disrupts immune and thrombogenic responses.[45] Obese people diagnosed with COVID-19 were more than twice likely to be hospitalized, 74% more likely to need an intensive care unit, and 48% more likely to die, according to a study from the University of North Carolina Research Group. According to a report from

China based on a systematic meta-analysis, comorbidities, including, obesity, hypertension, diabetes, cardiovascular disease, cerebrovascular disease (Metabolic Diseases), are clinical risk factors for severe are fatal outcomes associated with COVID-19, with obesity being the most prevalent.[46] In a series of articles, we and others have described the relationship between the underlying metabolic diseases and the severity of coronavirus disease.[33-42] We also have indicated that patients with metabolic diseases have a compromised vascular endothelium and hence the coronavirus diseases severity is much more severe than those who do not have any underlying conditions.[33-42] Thakur and associates did a systematic review and meta-analysis of data on 120 studies with 125,446 COVID-19 patients. The most prevalent comorbidity was hypertension (32%), obesity (25%), diabetes (18%), cardiovascular disease (16%). They also found that the association of comorbidities and severity of the disease varied in different geographical locations.[47]

Discussion

One can easily say that discovery of DNA structure and elucidation of the genetic code half a century ago, heralded the 'golden era' of biomedical research and innovation.[29] If one were to list the ten greatest medical milestones, then this discovery will be one of them. Then comes the flood of research in the human genome project—humanity's biggest research endeavor. Technology did not catch up with human aspirations and the quest for immediate cures for incurable diseases.

Then came the announcement of an ambitious project by the then US President, Barack Obama, the Precision Medicine Project. Dr. Francis Collins announced a billion-dollar program, "All of Us' with two main goals; a near-term focus on cancers and diabetes, and a longer-term aim to generate knowledge applicable to the whole range of health and disease. We have described such ambitious programs as 'top-down approaches' to find solutions. Whereas we and others have advocated a hypothesis-based approach to problems. When considering such approaches, the return on investment plays a big role. On the other hand, we cannot put a price on human lives. The speed at which we provide preventable or protective interventions could save millions of lives worldwide.

In brief, gene expression means manufacturing its corresponding protein, and this is a process that follows a specific set of events. In the primary step, the information in the DNA is transferred to a messenger RNA (mRNA) molecule by a process known as transcription. During this phase, DNA of the gene serves as a template for complementary base-pairing. The RNA polymerase catalyzes the formation of a pre-mRNA molecule, which is then processed to form a mature mRNA. The resulting mRNA is a single-stranded copy of the gene. "The development of RNA vaccines is a great boon to the future of treating infectious diseases," says Lynne Maquat, the J. Lowell Orbison Distinguished Service Alumni Professor in biochemistry and biophysics, oncology, and pediatrics at Rochester and the director of Rochester's Center for RNA Biology. Although these are the first mRNA vaccines to be approved for human use, the story of mRNA vaccines starts more than 30 years ago.

Dr. Kizzmekia Corbett of NIH was the inspiring researcher who helped create COVID-9 mRNA vaccines. In an exclusive interview with the Director of NIH, Dr. Francis Collins (June 17th, 2021), she explains how they went about developing a vaccine for COVID-19 so fast. She says, "messenger RNA technologies have been in development from a basic science perspective for over 15 years. Vaccines are basically to teach your immune system how to find the virus protein and attack. Dr. Corbett and associates have described the design, testing, and development of mRNA1273, which encodes SARS-CoV-2 spike proteins in the perfusion state.[48] In the MIT Technology Review, the author Regalado writes, "Scientists at Moderna were able to design a vaccine on paper 48 hours post announcement of the information of SARS-CoV-2 genome, 11 days before the US had its first COVID positive case. In the NIH interview posted on June 17th, 2021, Dr. Corbett says, "The cool thing about this type of technology is you don't even need the lab to design the vaccine." However, according to Dr. Corbett, RNA technologies have been in progress for over a decade. It took several months to scale up the process, do the clinical trials and manufacture doses of vaccines that could be authorized for emergency use.

Despite the availability of knowledge about the exact sequence of nucleotides needed to provide the information to the immune cells to make antibodies to Spike proteins of SARS-CoV-2, all attempts to deliver this messenger RNA into humans would have failed. The human immune system would have recognized the "foreign' molecule and destroyed it. Credit for the development of an appropriate delivery system with lipid nanoparticles goes to Katalin Kariko (now at BioNTech) and Drew Weisman of the University of Pennsylvania for the discoveries the pair made two decades ago.[49]

Various versions of lipids such as ionizable lipid nanoparticles can be used to safely deliver the mRNA to target cells. Özlem Türeci, German biotechnology company BioNTech's chief medical officer, and her colleagues optimized a therapy with what she describes as "different liposomal formulations to make RNA fit for the respective purposes like an intramuscular or intravenous injection and targeting specific cell types." BioNTech found that for anti-cancer vaccines based on liposomally formulated mRNA, for instance, the antigen is expressed mainly in the dendritic cells in lymphatic compartments. These cells specialize in setting off antigen-specific immune responses.

Each improvement made through emerging basic sciences and applied technologies improves the formulation, and offers less inflammation, enhanced expression, protected delivery of the mRNA molecules, and thus allows the immune-engineers to build better mRNA vaccines as therapeutics for viral diseases as well as for cancer.[50-52]

Conclusion

Biomedical innovations and technological advances have contributed significantly to our understanding of the mechanisms that induce dysfunction of various systems and

initiate the development of risk factors or promote diseases. As we have mentioned earlier, from discovery to the application of the knowledge for practical use takes considerable time. However, an unprecedented pandemic of coronavirus exposed the weakness of global medical emergency management. This unprecedented pandemic also promoted the warp-speed development of effective vaccine candidates. The FDA has approved 3 vaccines for emergency use, as well as some experimental drugs, such as the monoclonal antibody cocktail of Regeneron.

We have discussed how the breaking of the genetic code half a century ago and understanding the role of nucleotide sequences in the synthesis of appropriate, specific proteins of biological importance accelerated the studies on RNAs in general as well as on microRNAs and messenger RNAs. For the first time in half a century, scientists and biotechnologists were able to utilize the various pieces of information available and package the appropriate sequence of mRNA, which can code for the spike proteins of SARS-CoV-2 and develop a safe vaccine against COVID-19. This success, to a great extent, is the result of extensive studies on mRNAs of other pathogenic viruses. Credit also goes to the extensive studies that developed needed technologies for packaging of biological molecules in the appropriate lipid nanoparticles for targeted delivery to the desired tissue, cells, or organs.

The extraordinary success of mRNA vaccines has opened new avenues for mRNA-based therapies. mRNAs, siRNAs, and non-coding miRNAs will play a very important role as novel therapeutics soon. Furthermore, this success has acted as a catalyst for ongoing work on the use of small RNAs for therapeutic purposes. For instance, siRNAs have become an exciting tool not only in molecular biology but also in molecular medicine. According to the experts, miRNAs regulate more than a third of all cellular mRNAs, and bioinformatic data indicate that each miRNA can control hundreds of gene targets. Small non-coding RNAs could emerge as novel therapeutics soon. The success of mRNA vaccines has opened new avenues for genetic information coders, synthetic chemists, immunologists, virologists, biotechnologists, experts in engineering targeted delivery systems, and various stakeholders of drug discovery and development. Having said that, I must emphasize that there are many challenges and abundant opportunities.

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REVIEW ARTICLE

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Phosphorylation and Fragmentation of the Cardiac Troponin T: Mechanisms, Role in Pathophysiology and Laboratory Diagnosis

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Abstract

Cardiac troponin T (cTnT), a protein essential for calcium-regulated, myofibrillar ATPase activity, is extremely sensitive to the action of a significant number of intra- and extracellular enzymes, the action of which causes post-translational modifications (PTMs) of amino acid structure and functioning cTnT. PTMs of cTnT may play important roles in the regulation of cardiac contractility. The vast majority of cTnT modifications involve the phosphorylation by a variety of Ser/Thr kinases, including PKC. At the same time, the activity of cTnT phosphorylation can change under physiological conditions and in some cardiovascular diseases, including heart failure, acute myocardial infarction, and arrhythmias. Along with cTnT phosphorylation, cTnT fragmentation occurs, the activity of which can also change. This article discusses the mechanisms of cTnT phosphorylation and fragmentation, discusses the important role of these processes in the pathophysiology and laboratory diagnosis of some cardiovascular diseases, and notes promising directions for further research. (**International Journal of Biomedicine. 2021;11(3):250-259.**)

Key Words: cardiac troponin T • phosphorylation • fragmentation • cardiovascular disease

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Abbreviations

AF, atrial fibrillation; **AMI**, acute myocardial infarction; **ATP**, adenosine triphosphate; **ASK1**, apoptosis signal-regulating kinase 1; **AF**, atrial fibrillation; **CVD**, cardiovascular disease; **cTnT**, cardiac troponin T; **HF**, heart failure; **LV**, left ventricle; **LVR**, left ventricular remodeling; **LVH,** left ventricular hypertrophy; **PTMs**, post-translational modifications; **PMA**, phorbol 12-myristate 13-acetate; **PKC**, protein kinase C; **PAK1**, p21-activated kinase 1; **PP2A**, protein phosphatase 2A; **ROCK,** Rho-A–dependent kinase; **RV**, right ventricle; **Tn**, troponin; **TnT**, troponin T; **Tm**, tropomyosin.

Troponin (Tn) is essential in Ca^{2+}-activated contraction of skeletal and cardiac muscles. Tn consists of three subunits (TnT, TnC and TnI) and, together with Tm, is located on the actin filament.(1) TnC, the $Ca2^{+}$ binding subunit, transduces Ca^{2+} signaling; TnI, the inhibitory subunit, inhibits myosin ATPase activity; and TnT, the tropomyosin binding subunit, anchors the Tn protein complex to the thin filament.(1) cTnT plays a pivotal regulatory role in the Ca^{2+}-mediated interaction between actin thin filament and myosin thick filament. At low cytosolic Ca^{2+} levels, the formation of the actomyosin complex is sterically inhibited by TnI. The resting inhibitory state is rapidly transformed by the 100-fold increase in intracellular Ca^{2+} concentration occurring as a consequence of sarcolemmal depolarization. At an increased cytosolic level, Ca^{2+} binds to TnC and induces a sequence of conformational changes in the Tn–Tm complex that exposes the specific myosin-binding site on actin. Tm normally sterically blocks the interaction between myosin heads and actin. When TnC is saturated with Ca^{2+}, the inhibition of myosin binding to actin by tropomyosin is reversed. This is apparently due to the small movement of Tm,

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caused by the dimensional changes in TnC upon Ca^{2+} binding that remove a steric block. The result is that myosin heads are able to contact actin, with formation of active actin-*myosin* cross-bridges and generation of contraction (Fig.1). [2,3]

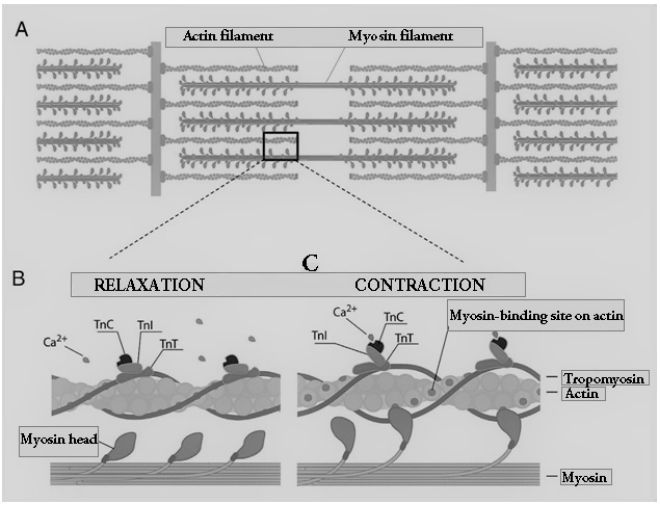

Fig.1. *The structure and conformational changes in the Tn–Tm complex during contraction and relaxation*

A – Sarcomere structure; B –Structure of a thin filament in the relaxation of cardiomyocytes; C – Structure of a thin filament in the contraction of cardiomyocytes

As a rule, the more Ca^{2+} that is able to bind to the thin filament, the higher the contraction force. An increase in the contractile ability of the heart can also lead to a higher force of contraction and allows more blood to be ejected during one stroke volume.[4] Myofilaments have a variable sensitivity to Ca^{2+}, which affects the binding of cTnC to Ca^{2+} ions and, accordingly, the contractility of the heart muscle. The higher sensitivity of myofilaments to Ca^{2+} leads to an earlier contraction in systole and a slower relaxation in diastole. Energy for heart contraction is provided by ATP, which is hydrolyzed by the enzyme ATPase in the myosin head. The enzymatic activity of ATPase correlates with the rate of the formation of cross-bridges and shortening of the sarcomere, the rate of energy consumption by cardiomyocytes, and with the average heart rate in different species.[5,6]

PTMs of key contractile proteins play an important role in regulating cardiac output and maintaining it in accordance with the body's needs. The most significant of these are the processes of protein phosphorylation under the influence of certain enzymes called kinases. Under the action of kinases, proteins are mainly phosphorylated on the following amino acids: serine (Ser), threonine (Thr), and tyrosine (Tyr). The exact molecular mechanisms of phosphorylation processes, as well as their effect on cardiac contractility, are still not fully understood. Researchers continue to discover new sites of phosphorylation and kinases, which not only play a physiological role, but are also of great importance in the pathogenesis of CVD (HF, AMI, and AF).[7]

Another important but less studied type of PTM is the cTnT fragmentation. The study of this process is important for the pathogenesis and laboratory diagnosis of CVD. Under the conditions of various cardiac and non-cardiac pathological conditions,[8-10] which have an adverse effect on cardiomyocytes, the activity of proteolytic enzymes that cause the cleavage of cTnT into fragments can be enhanced. The development of antibodies directly to these fragments can increase the sensitivity of existing immunoassays and improve the early diagnosis of CVD. In addition, the size of these fragments is so small that it allows them to pass through structural components into other biological fluids, for example, saliva and urine, as has recently been demonstrated in several studies.[11-13]

This review examines the mechanisms of post-translational phosphorylation and fragmentation of cTnT, and discusses the importance of these processes in the physiology and pathophysiology of cardiac muscle contractions, as well as in clinical laboratory diagnostics.

Troponin T isoforms

Human TnT is encoded by three homologous genes (TNNT2, TNNT1, and TNNT3) and expressed as three isoforms: cardiac (cTnT), slow skeletal (ssTnT), and fast skeletal (fsTnT) muscle TnT, respectively.[14,15]

The three TnT isoforms are significantly diverged in the N-terminal region but highly conserved in the middle and C-terminal regions that contain binding sites for TnC, TnI and Tm.[14,16]

In the studies of Anderson et al.,[17] it has been shown that cTnT is expressed in the human heart as four isoforms ($cTnT_1$ through $cTnT_4$, numbered in the order of decreasing molecular size). cTnT1 and $cTnT_2$ are expressed in the fetal heart, with $cTnT_2$ being expressed at a very low level. $cTnT_4$ is expressed in the fetal heart and is re-expressed in the failing adult heart, whereas $cTnT_3$ is the dominant isoform in the adult heart.[17-19]

Phosphorylation of cTnT: mechanisms, role in the physiology and pathophysiology of CVD

Phosphorylation is a fundamental mechanism in regulating the structure and function of cTnT. The earliest reports of cTnT phosphorylation date back to the 1980s, when the research groups of Villar-Palasi et al.[20] and Guseva et al.[21] identified certain features for cTnT phosphorylation, but researchers were unable to identify the enzyme responsible for this reaction. Subsequently, it was found that PKC is responsible for the phosphorylation of cTnT, and this was finally confirmed by in vitro studies.[22,23] Noland et al.[22] and Swiderek et al.[23] incubated cTnTs derived from bovine myocardium with PKC for various periods of time. Researchers reported phosphorylation of various regions of cTnT after prolonged incubation with PKC. Using protein sequencing according to Edman's method, it was revealed that in addition to the Ser-2 site, multiple Ser and Thr residues (Thr204, Ser208, Thr213 and Thr294) are also phosphorylated by protein PKC, especially PKCα, PKCε and PKCξ.[16,24-25] Noland and Kuo[26] showed that the Ca^{2+}-stimulated MgATPase of actomyosin containing phosphorylated cTnT, compared with that containing unphosphorylated TnT, was decreased by up to 48%. Phosphorylation of cTnT also decreased (up to 48%) its maximum binding to Tm-F-actin. The authors concluded that

the effects of phosphorylated TnT in decreasing actomyosin MgATPase might be secondary to its decreased interactions with the other components of the thin filament.

Several studies have investigated PKC-mediated, site-specific effects of cTnT phosphorylation. Thr197, Ser201, Thr206 and Thr287 in the C-terminal region of cardiac TnT were identified as functionally important PKC phosphorylation sites.[27-32] Substitution of the Ser or Thr residue with Glu to mimic the negative charge introduced by PKC phosphorylation of cardiac TnT caused decreases in maximum force development and calcium sensitivity. M. Sumandea et al.[31] found that Thr206 is a functionally critical cTnT PKC phosphorylation residue. Its exclusive phosphorylation by PKC-α or replacement by Glu (mimicking phosphorylation) significantly decreased maximum tension, actomyosin Mg-ATPase activity, myofilament Ca^{2+} sensitivity, and cooperativity. It was also observed that PKC dependent phosphorylation of Thr206alone was sufficient to reduce maximum tension development.[24]

The PKC family consists of a number of different isozymes with different substrate specificities.[33,34] Classical PKCs (isoforms α, β1, β2, and γ) are activated by phosphatidylserine, Ca^{2+}, and diacylglycerol (or PMA). Novel PKCs (δ, ε, η, θ, and μ) are not activated by $Ca2^{+}$ but are activated by PMA and diacylglycerol. The atypical PKCs (ζ, ι, and λ) are not activated by Ca^{2+}, PMA, or diacylglycerol. [35,36] PKCα directly phosphorylates regulatory myofilament proteins such as cTnI and cTnT.[30] cTnT and cTnI also serve as targets for PKCε.[37] Jideama NM et al. [30] showed that PKC isozymes α, δ, ϵ, and ζ displayed distinct substrate specificities in phosphorylating TnI and TnT subunits in the bovine cardiac troponin complex. Thus, PKC-α, -δ, and -ϵ phosphorylated TnI more than TnT, but PKC-ζ conversely phosphorylated the latter more than the former.

However, S. Wu and R. Solaro [38] identified the atypical PKCζ isoform to associate specifically with cTnI in untreated adult rat ventricular cardiac myocytes. According to several studies, the most common PKC isoform present in adult ventricular myocytes, PKCε, interacts with both cTnI and cTnT.[37,39-41]

The various effects of PKC isoforms can be attributed to several factors. First, some PKC isoforms have a number of other intracellular targets, the activation of which can subsequently neutralize the main phosphorylating effect of PKC on cTnT and cTnI. Thus, it was shown that PKCζ is also involved in the Pak1/PP2A pathway leading to Thr dephosphorylation of cTnI and cTnT.[38-41] These data indicate that the activation of PKCζ is a significant control mechanism regulating both phosphorylation and dephosphorylation of myofilament proteins. Second, the authors used various methods of phosphorylation, which could also affect the results obtained. In particular, in vitro phosphorylation is based on incubation in buffers containing purified protein kinases but lacking other components—other intracellular kinase targets and dephosphorylation components (phosphatase enzymes). In situ studies are carried out by activating the expression of phosphorylation enzymes in viable cardiomyocytes, in which many other kinase targets are present. This may be the main reason for the opposite results, such as a decrease in phosphorylation of troponin molecules. As shown in a study by Wu et al.,[38] protein kinases activate additional targets and phosphatases. Dephosphorylation can work as a compensatory mechanism to reverse the decreased contractility resulting from the phosphorylation of cTnT and cTnI.

Jideama et al.[30] discovered unique evidence that PP1, a serine/threonine protein phosphatase, effectively dephosphorylated TnT and TnI in the thin filament. The authors revealed that while PKC and PKA phosphorylation decreased the Ca^{2+}-stimulated Mg^{2+}ATPase activities of the rat cardiac myofibrils, PP1 dephosphorylation restored it close to that of the control values. PP1 does not have a specific target site for dephosphorylation of cTnT; however, the Thr-213 site is the least sensitive to the action of PP1, while the PP2A enzyme specifically targets it,[36,42] which suggests a different role for the two phosphatases. Based on the above, it becomes obvious that the phosphorylation-dephosphorylation reactions are subject to fine regulation to maintain the optimal contractile function of the myocardium and the corresponding oxygen requirements of the body.

It has also been reported that kinases other than PKC are also involved in phosphorylation of cTnT. ASK1, highly expressed in cardiac muscle, is an important mediator in the signaling pathways induced by tumor necrosis factor interleukin-1, and ROS.[43,44] He et al.[44] showed that ASK1 plays an important role in the regulation of cardiac contractile function by phosphorylating cTnT and may participate in cytokine/ROS-induced pathogenesis of cardiomyopathy and heart failure. In particular, ASK1 phosphorylates cTnT at sites T194 and S198 within an ASK1 consensus phosphorylation sequence (although other sites may also be phosphorylated). Vahebi et al.[45] found that ROCK-II induced a depression in maximum ATPase rate and tension, which was associated with phosphorylation of TnT, TnI, and myosin-binding protein C. Mass spectrometric analysis demonstrated that ROCK-II phosphorylated cTnI at S23, S24, and T144 and cTnT at S278 and T287 sites. Pfleiderer et al.[46] showed that Raf-1, a serine-threonine protein kinase**,** acts as a selective cTnT-Thr^{206} kinase; Raf does not phosphorylate cTnI. These data identify Raf-dependent cTnT-Thr206 phosphorylation as a novel mechanism that would link growth factor-dependent signaling pathways to dynamic changes in cardiac contractile function. Table 1 summarizes the known data on cTnT phosphorylation.

Phosphorylation of cTnT may play an important role in the pathophysiology of CVD and in laboratory diagnosis. Given that the in vitro-measured effects of cTnT phosphorylation usually indicate a decrease in myofilament contractility (Table 1) and that PKC activity increases in response to hypertrophic signaling and in heart failure,[47,48] it can be assumed that cTnT phosphorylation is a characteristic feature of these pathological conditions. Belin et al.[49] studied molecular mechanisms that explain interventricular differences in myofilament function in experimental congestive HF induced in rats. PKC-α-dependent phosphorylation of cTnI and cTnT was greater in failing LV myofilaments than in failing RV myofilaments. In failing RVs, total cTnI and cTnT phosphoprotein levels were significantly increased by ~50%, relative to controls (P<0.05).

In failing LVs, total cTnT and cTnI phosphorylation levels were increased by ~50% (P=0.053) and 102% (P<0.05), respectively, relative to controls. Phosphorylation of cTnT at Thr206 was increased 87% in failing RV muscles and 24% in failing LV muscles, compared to control.

A number of researchers have conducted several studies to analyze the phosphorylation of contractile proteins after AMI.[50-52] Using a rat model of MI and phosphoproteomic technology, Dubois et al.[53] discovered that remodeling is associated with decreased levels of myocardial and plasma Ser208-phosphorylated TnT. To confirm the association in human plasma, the new specific polyclonal antibodies against human/rat Ser(207/208)-phosphorylated TnT were used to test plasma obtained from patients in the first week after MI, with low, intermediate, and high LVR a year later. The study found a significant decrease of Ser207-phosphorylated TnT and of the Ser207-phosphorylated TnT/total TnT ratio in those with intermediate or high LVR. An increase in TnT phosphorylation was also found in AF.[54]

In addition, increased activity of PP1 and PP2A was also noted in patients with AF[54,55]. This may indicate the formation of a specific compensatory mechanism, namely, that the increased activity of PP1 and PP2A counteracts the effects of kinases (phosphotransferases). Table 2 summarizes the data on cTnT phosphorylation in pathological conditions.

Fragmentation of cTnT: mechanisms, significance in laboratory diagnosis and pathophysiology of CVD

cTnT is sensitive not only to the action of kinases and phosphatases, but also to the action of many proteases, which can change their activity under certain physiological and pathological conditions. Proteolytic modifications of cTnT and cTnI have been shown to have pathological effects on myocardial contractility.[56,57] Communal et al.[58] examined whether caspase-3 cleaved cardiac myofibrillar proteins and, if so, whether it affects contractile function. When cTnT, cTnI, and cTnC were incubated individually with caspase-3, there was no detectable cleavage. However, when the recombinant troponin complex was exposed to caspase-3, cTnT was cleaved, resulting in fragments of 25kDa. This destructive modification of cardiac TnT decreased the maximum myosin ATPase activity and myofibril force generation.[58]

Mu-calpain is a myofibril-associated protease and is known to degrade TnT. TnT is known as a protein with extended conformation, in which the NH2-terminal variable region is a part of the "tail" domain of troponin. This region does not contain binding sites for other thin-filament proteins, but alteration of its structure affects the Ca2+ regulation of muscle contraction.[59] A study on restricted proteolytic modification of cTnT represents a new area of research and will provide valuable information to further understand the role of post-translational regulation in cardiac muscle function and diseases. Zhang et al.[59] reported production of the NH2-terminal truncated cardiac TnT (cTnT-ND_{72-291}) during myocardial ischemia-reperfusion. Mu-calpain treatment of the cardiac myofibril and troponin complex specifically reproduced cTnT-ND. In contrast, mu-calpain treatment of isolated cardiac TnT resulted in nonspecific degradation, suggesting that this structural modification is relevant to the physiological structures of the myofilament.

Di Lisa et al.[60] showed that mu-calpain was at least ten times more active than m-calpain in degrading TnI and TnT both in vitro and in situ. It is interesting that phosphorylation by PKC resulted in a twofold increase in the degradation of TnI.

A restricted proteolysis of cardiac TnT was recently found [27] to be a novel regulatory mechanism in physiological and pathophysiological adaptations of the cardiac muscle. Different from the destructive cleavage by caspase 3, this restrictive proteolysis selectively removes only the N-terminal variable region and preserves the conserved regions of cardiac TnT. Experimental data have shown that selectively removing the N-terminal variable region does not destroy the function of TnT but alters the binding affinities for TnI, TnC and Tm.[61] Previous studies by several laboratories showed that selective removal of the N-terminal variable region of TnT slightly decreased the maximum myosin ATPase activity and myofibril force generation without affecting thin-filament calcium sensitivity and cooperativity.[27,62-64]

Cardiac necrosis in AMI is accompanied by the release of various proteolytic enzymes from lysosomes. It is generally accepted that cTnI is very sensitive to proteolysis. The appearance of immunoreactive cTnI fragments in human serum after AMI has been confirmed by several groups of researchers.[65-68] Degradation and changes in cTnI have implications for the immunoreactivity of antibodies used in various clinical analyses.[67-69] This leads to different results when measuring the same serum sample with different cTnI immunoassays that have different anti-cTnI antibodies, which complicates the clinical interpretation of these measurements.

cTnT is an acknowledged biomarker of AMI that is known to be prone to proteolytic degradation in serum. Several studies devoted to the analysis of cTnT from serum samples of AMI patients revealed a set of proteolytic fragments with apparent molecular masses of 29, 19, 18, and 16-kDa, with the 29-kDa fragment being the predominant form.[70-74] Fragmentation of cTnT in the blood serum of AMI patients was shown by different groups using gel filtration chromatography [68] and immunoblots.[70,73,75]

Cardinaels et al.[73] demonstrated that the Roche cTnT immunoassay detects intact as well as degraded cTnT forms in AMI patients' sera during the period of diagnostic testing. Intact cTnT rapidly disappears from the circulation during the early hours after AMI, but immunoreactive fragments remain present longer. These results are consistent with Michielsen et al.,[70] who found that intact cTnT rapidly disappears from the circulation during the early hours after AMI, but immunoreactive fragments remain present longer.

Extensive fragmentation of cTnT has also been found in the serum of patients with end-stage chronic renal failure.[71,72] It is likely that in chronic renal failure these fragments accumulate due to a decrease in clearance, which may lead to an overestimation of the cTnT concentration.[71,76,77] In the multi-ethnic Chronic Renal Insufficiency Cohort (CRIC), high sensitivity (Hs)-TnT was detectable in 81% of subjects. In addition, lower eGFR was associated with higher expected hs-TnT. Pervan et al.[11] showed that kidneys are the main organ of elimination of troponin from blood.

Table 1.
cTnT phosphorylation: mechanisms and physiological effects

Phosphorylation site	Enzyme	Object and type of study	Physiological effect	Source
Thr190, Thr199, Thr280	PKC	Bovine heart, in vitro	Not studied	[22]
Thr 190, Thr 194, Thr 199, Thr 280	PKC-α, PKC-δ, PKC-ε, PKC-ζ	Bovine heart, in vitro	Phosphorylation of TnT by PKC-α yielded marked decreases in both Ca2+ sensitivity and activity of MgATPase. Phosphorylation by PKC-ζ at distinct, unknown sites resulted in a slightly increased Ca2+ sensitivity without affecting the activity of MgATPase.	[30]
Thr 194, Ser 198	ASK1	Human heart, in vitro / Rat heart, in situ	Overexpression of ASK1 induces cTnT phosphorylation and inhibits contractility in cardiomyocytes	[44]
Thr197, Ser201, Thr206, Thr287	PKC-α	Mouse heart, in vitro	Thr206 exclusive phosphorylation by PKC-alpha significantly decreased maximum tension, actomyosin Mg-ATPase activity, myofilament Ca2+ sensitivity, and cooperativity.	[31]
Thr206	Raf-1	Rat heart, in vitro	Raf-dependent cTnT-Thr206 phosphorylation was found to be a novel mechanism that would link growth factor-dependent signaling pathways to dynamic changes in cardiac contractile function.	[46]
Ser278, Thr287	ROCK-II	Mouse heart, in vitro	A depression in maximum ATPase rate and tension	[45]

Table 2.
Data on cTnT phosphorylation in pathological conditions

Pathological condition	Object and type of study	Changes in cTnT phosphorylation and possible effects	Source
HF	Failing human LV tissue	The altered thin-filament function in human failing myocardium was associated with PKC-mediated phosphorylation of TnT.	[47]
HF	Mouse model	The prolonged effect of PKCε overexpression for 6 months brings a decrease in the Ca2+ sensitivity of the myofilaments. The decrease in Ca2+ sensitivity correlates with increased cTnI/cTnT phosphorylation.	[48]
Congestive HF	Rat model	Expression and activation of PKC-α was increased twofold in failing RV myocardium and relative to the RV. Phosphorylation of cTnI and cTnT by PKC-α was greater in failing LV myofilaments than in failing RV myofilaments.	[49]
AMI	Pig model	No differences in cTnT phosphorylation were found between sham and MI hearts	[52]
AMI	Plasma of patients with AMI	A decreased TnT- Ser207-phosphorylation was found in patients with high LVR after AMI	[53]
AF	Cardiomyocytes from human right atrialappendages	An increase in TnT phosphorylation was found in AF	[54]

Table 3.
Data on the pathophysiological and laboratory significance of PTMs (phosphorylation and fragmentation) in CVD

PTM	Pathophysiological and laboratory significance	Source
TnT phosphorylation and CVD pathophysiology	PKC-dependent phosphorylation of cTnT was found to be increased in cardiac hypertrophy and HF	[47], [48], [49]
	A decreased TnT-Ser207-phosphorylation was found in patients with high LVR after AMI	[53]
	An increase in TnT phosphorylation was found in AF. Increased activity of PP1 and PP2A was noted in patients with AF that may indicate the formation of a specific compensatory mechanism, namely, that the increased activity of PP1 and PP2A counteracts the effects of kinases.	[54,55]
TnT phosphorylation and Lab tests in CVD	LVR wass associated with decreased levels of myocardial and plasma Ser208-phosphorylated TnT.	[53]
Fragmentation of cTnT and Lab tests in CVD	Fragmentation of cTnT in the blood serum of AMI patients was shown by different groups using gel filtration chromatography and immunoblots.	[68], [70], [73], [75]
Fragmentation of cTnT and Lab tests in CVD	The 29-kDa fragment of cTnT in AMI serum samples mainly appears due to the cleavage by thrombin during serum sample preparation.	[78]

Along with the well-studied cTnT degradation by the action of mu-calpain, there are other candidates for that role. The results of the study by Katrukha et al.[78] suggest that the 29-kDa fragment of cTnT in AMI serum samples mainly appears due to the cleavage by thrombin during serum sample preparation. Apart from thrombin, some other protease(s) cause the further degradation of the 29-kDa fragment to form 16–19-kDa fragments in serum.[73,74] Katrukha et al.[78] highlighted that the knowledge of sites of cTnT degradation is very important, both (a) for the selection of the antibodies that are not affected by thrombin-mediated cTnT proteolysis; and (b) for the selection of the proper matrix to be used for cTnT measurements. The development of immunoassays specifically aimed at detecting intact, fragmented, or phosphorylated cTnT can help in studying the pathophysiology of degradation of cTnT and, accordingly, lead to improved laboratory diagnosis of CVD.[79-86]

Table 3 summarizes the data on the pathophysiological and clinical and laboratory significance of PTMs (phosphorylation and fragmentation) of cTnT.

Conclusion

The Tn–Tm complex is an important regulatory protein complex that is required to maintain the contractile ability of the heart. The Tn–Tm complex changes dynamically and adapts to meet the necessary needs of the body. According to most studies, phosphorylation of cTnT reduces the activity of ATPase, decreases the maximum tension of myofilaments and decreases sensitivity to Ca2+ ions, which leads to a decrease in myocardial contractility. Changes in cTnT phosphorylation may play an important role in the pathogenesis of CVD, including HF, AMI, and AF. Thus, in HF, there is an increased expression of PKC and a subsequent increase in cTnT phosphorylation, which ultimately leads to a gradual decrease in myocardial contractility. Quite remarkable are the observations that the phosphorylation of cTnT in the heart correlates with the phosphorylation of circulating cTnT in plasma after AMI, which may be a predictor of the development of LV remodeling and can be used as such in laboratory diagnostics. Because of their cardiac specificity, cTnI and cTnT are actively used as biomarkers of myocardial alteration in AMI and HF. One of the promising areas for further research is the processes of fragmentation of cTnT into smaller fragments that can pass through hematotissue barriers into biological fluids obtained by non-invasive methods (urine and saliva), which will help expand the pool of specific immunoassays with high diagnostic value.

Competing Interests

The author declares that there is no conflict of interest regarding the publication of this article.

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ORIGINAL ARTICLE

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Evaluation of the Prognostic Value of Heart Rate Variability in Elderly Patients with Multivessel Coronary Artery Disease against the Background of Invasive and Non-Invasive Treatment

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Abstract

The purpose of this study was **t**o evaluate the prognostic value of heart rate variability (HRV) in elderly patients with multivessel coronary artery disease on the background of invasive and non-invasive treatment.

Methods and Results: This study included 254 patients over age 65 with lesions of the left trunk of the left coronary artery in combination with lesions of 2 or more coronary arteries. To assess HRV, all patients underwent 24-hour Holter ECG monitoring at baseline and one year later. Depending on the treatment strategy, patients were divided into 3 groups. Group 1 consisted of 99 patients who, in addition to the standard treatment, underwent percutaneous coronary intervention (PSI) (from 1 to 4 stents); Group 2 included 86 patients who, in addition to the standard treatment, underwent coronary artery bypass grafting (CABG) (from 2 to 4 shunts); Group 3 included 69 patients who received only optimal drug therapy (ODT). The results have shown that a decrease in HRV is an independent predictor of complications associated with an increase in coronary insufficiency in CAD patients. Such indicators of HRV as SDNN, SDNNi, TP, VLF, and LF have a significant positive predictive value in patients undergoing ODF and/or undergoing PCI. For patients undergoing CABG, at least in the first year after surgery, HRV cannot be considered as an independent prognostic marker. For elderly patients with multivessel coronary artery disease, 24-hour Holter ECG monitoring with subsequent analysis of HRV is recommended to assess the recovery process and pharmacotherapy. **(International Journal of Biomedicine. 2021;11(3):260-264.)**

Key Words: coronary artery disease • heart rate variability • PSI • CABG • drug therapy

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Abbreviations

ANS. autonomic nervous system; **CABG**, coronary artery bypass grafting; **CAD**, coronary artery disease; **HR**, heart rate; **HRV**, heart rate variability; **HF**, heart failure; **LVEF**, left ventricular ejection fraction; **LCA**, left coronary artery; **ODT**, optimal drug therapy; **PCI**, percutaneous coronary intervention; **SD**, sudden death.

Introduction

Coronary artery disease (CAD) remains the most common human disease, and the mortality it causes is higher than all other causes of death.[1] Patients with CAD are a rather heterogeneous group; more than half of them have a multivessel lesion, that is, they represent a complex and severe category of patients. Along with the development of the therapeutic direction of treatment, there is a continuous development of surgical methods for the treatment of CAD. The modern direction of surgical interventions involves both minimally invasive percutaneous coronary intervention (PCI) and open

heart surgery (coronary artery bypass grafting [CABG]). In the light of the constant improvement and development of new directions in CAD treatment, it is relevant to identify various prognostic markers that make it possible to assess the course of the disease, especially since some of the most commonly used indicators, for example, the ejection fraction, in some cases may be insufficiently informative and may not correlate with the severity of the clinical condition, especially with the progression of coronary atherosclerosis.[2]

Undoubtedly, dysfunction of the central and autonomic nervous systems, along with heredity and endocrine-metabolic imbalance, is a significant factor affecting the development of CAD. The sympathetic/parasympathetic imbalance leads to an increase in the inflammatory status in the cardiovascular system and biotransformation of atherosclerotic plaques with the development of coronary complications.[3]

It should be remembered that the parasympathetic system directly affects the electrophysiological properties of the myocardium, and can also inhibit adrenergic activity.[4] One of the effective and available methods for studying the state of the autonomic nervous system (ANS) is the analysis of heart rate variability (HRV). This method allows us to assess the state of both sympathetic and parasympathetic divisions of the ANS. At the same time, low HRV correlates to a greater extent with the risk of sudden death (SD) than some generally accepted indicators of the clinical severity of the patient's condition: LVEF, ventricular arrhythmias, and degree of exercise tolerance.[3,5,6] With aging, the autonomic effect on the cardiovascular system weakens, and the autonomic regulation of the heart gradually disintegrates. This fact correlates with the results of experimental studies, which have shown a weakening of functional connections between parts of the central nervous system in aged animals.[4,7] The data obtained in a number of studies [7,8] also indicate a weakening of the effect of the ANS on the cardiovascular system with age.[9-11]

The purpose of this study was **t**o evaluate the prognostic value of HRV in elderly patients with multivessel coronary artery disease on the background of invasive and non-invasive treatment.

Materials and Methods

This study included 254 patients over age 65 with lesions of the left trunk of the LCA in combination with lesions of 2 or more coronary arteries. All patients received the standard basic therapy (ODT): antiplatelet therapy (acetylsalicylic acid, clopidogrel), β-blockers (bisoprolol), ACE inhibitors, statins (rosuvastatin).

Depending on the treatment strategy, patients were divided into 3 groups. Group 1 consisted of 99 patients who, in addition to the standard treatment, underwent PSI (from 1 to 4 stents); Group 2 included 86 patients who, in addition to the standard treatment, underwent CABG (from 2 to 4 shunts); Group 3 included 69 patients who received only ODT.

Based on the results of one-year follow-up, each group was divided into 2 subgroups: (A) those who reached at least one endpoint and (B) those who did not reach the endpoints. The following endpoints were studied: lethal outcome, myocardial infarction (non-fatal), progression of coronary insufficiency, development and progression of heart failure (HF), repeated hospitalizations associated with an increase in coronary insufficiency, the need for surgery.

To assess HRV, all patients underwent 24-hour Holter ECG monitoring at baseline and one year later. Key indicators:

mRR (ms), the average duration of all RR intervals

SDNN (ms), the standard deviation of NN intervals

SDNN index (SDNNi) (ms), the mean of the standard deviations of all the NN intervals for each 5 min segment of a 24 h HRV recording

RMSSD (ms), the square root of the mean squared difference between adjacent R-R intervals

pNN50, the percentage of successive RR intervals that differ by more than 50 ms

HF power (ms^2), the absolute power of the high-frequency band (0.15–0.4 Hz); LF power (ms^2), the absolute power of the low-frequency band (0.04–0.15 Hz)

VLF power (ms^2), the absolute power of the very-low-frequency band (0.0033–0.04 Hz)

ULF power (ms^2), the absolute power of the ultra-low-frequency band (≤0.003 Hz)

TP, total power (ms^2)

LF/HF, the ratio of LF-to-HF power

The results were evaluated using the CardioSens+v.3.0 (KhAI Medica, Ukraine).

Statistical analysis was performed using the statistical software «Statistica». (v6.0, StatSoft, USA). For all types of analysis, the value of $P \leq 0.05$ was considered statistically significant.

Results

In Group 1 (PCI group), there were no differences in HRV between subgroups at baseline. At the same time, in both subgroups, baseline SDNN <50 ms were noted. A number of studies have shown that a decrease in SDNN less than 50 ms has a predictive value for detecting ventricular arrhythmias and SD risk,[6,12] as well as the LF/HF ratio >1.5, which indicates a shift in the autonomic balance towards an increase in sympathetic tone.[13]

In the dynamics, there were significant differences in the time-domain HRV indicators between subgroups. In particular, we found a significant increase in night-time SDNN in Subgroup B, compared to Subgroup A (68.3±10.8 ms vs. 43.2±8.8 ms, P=0.000). Frequency-domain HRV indicators were also significantly better in Subgroup B than in Subgroup A (LF power: 995±274.5 ms^2 vs. 359.8±151.4 ms^2, P=0.000), which indicates an increase in the sympathetic tone of the ANS. In general, in Subgroup A, the HRV indicators did not change during one year, compared to Subgroup B, with a positive dynamic of both time-domain and frequency-domain HRV indicators (Table 1). In Subgroup B, we found a decrease in the night-time HR from 76.2±6.1 bpm to 60.3±3.6 bpm (P=0.01), an increase in mRR from 765.5±8.5 ms to 1005.5±70 ms (P=0.01).

Table 1.

HRV indices in PCI group

Indicator	Subgroup A (n=57)			Subgroup B (n=42)			P_{A-B}
	baseline	one-year follow-up	*P*	baseline	one-year follow-up	*P*	
mRR, ms	868 ±146	916.5 ±64.2	0.8	765.5 ±8.5	1005.5 ±70	0.01	0.000
SDNN, ms	36.5 ±12.5	43.2 ±8.8	0.69	29.5 ±3.5	68.3 ±10.8	0.01	0.000
SDNNi, ms	36.9 ±12.4	39.1 ±11.5	0.89	29.2 ±3.3	63.9 ±9.4	0.01	0.000
TP, ms^2	1470.5 ±886.5	1900.7 ±717.2	0.71	843 ±188	3988.5 ±1129.8	0.02	0.000
VLF, ms^2	617.5 ±352.5	927.5 ±282.3	0.54	396 ±69	1280.8 ±364.8	0.02	0.000
LF, ms^2	430 ±329	359.8 ±151.4	0.87	230 ±90	995 ±274.5	0.02	0.000
HF, ms^2	210 ±162	44 ±18.6	0.53	60.5 ±37.5	40 ±13.6	0.22	0.241
LF/HF	2.2 ±0.2	4.6 ±4.8	0.39	4.7 ±1.4	1.9 ±1.04	0.25	0.000

P_{A-B} - between subgroups one year after PCI

Table 2.

HRV indicators in the CABG group

Indicator	Subgroup A (n=52)			Subgroup B (n=34)			P_{A-B}
	baseline	one-year follow-up	*P*	baseline	one-year follow-up	*P*	
mRR, ms	878.5 ±94.7	929.4 ±91.3	0.78	979 ±121	935.4 ±82.7	0.78	0.758
SDNN, ms	34.6 ±7.5	44.3 ±10.9	0.59	59.5 ±24.5	43 ±4.3	0.62	0.510
SDNNi, ms	38.5 ±8.3	44.2 ±10.9	0.82	59.8 ±24.4	44.6 ±5.7	0.65	0.844
TP, ms^2	1549 ±783.5	2017.6 ±977.8	0.72	4075 ±2833	1992.5 ±507.6	0.6	0.891
VLF, ms^2	717.4 ±264.8	1014.4 ±496.5	0.46	1615 ±940	984.8 ±274.9	0.62	0.752
LF, ms^2	426.3 ±128.8	336.3 ±130.9	0.82	1070.5 ±896.5	389.1 ±187.4	0.59	0.128
HF, ms^2	184.5 ±68.2	272.7 ±177	0.62	621 ±578	195.8 ±132.1	0.6	0.033
LF/HF	2.1 ±0.6	1.9 ±0.8	0.29	2.9 ±1.3	2.6 ±1	0.88	0.001

P_{A-B} - between subgroups one year after CABG

A significant increase in such indicators as SDNN, including SDNNi (from 29.2±3.3 ms to 63.9±9.4 ms, *P*=0.01), TP (from 843±188 ms^2 to 3988.5±1129.8 ms^2, *P*=0.02), and VLF power from 396±69 ms^2 to 1280.8±364.8 ms^2, *P*=0.02) indicates a reliable predictive value of the HRV changes (Table 1).

In Group 2 (CABG group), there were no differences between subgroups, both at baseline and one year later (Table 2). In generally, the dynamics of HRV indices was not so pronounced, which, most likely, is associated with surgical intervention that disturbs the neurohumoral relations and a relatively short follow-up period. Previously, it was shown that one year after CABG, HRV parameters remain sharply reduced, while in patients with CABG performed 3 years ago, the values of the main HRV parameters are close to patients with normal HRV parameters, i.e. there is a certain restoration not only of the coronary circulation but also of the neurohumoral relations.[14]

In Group 3 (ODT group), there were no baseline differences in HRV in subgroups. It should be noted, however, there was a general decrease in HRV. In dynamics, the best HRV indicators were observed in Subgroup B (Table 3).

Table 3.

HRV indices in ODT group

Indicator	Subgroup A (n=46)			Subgroup A (n=23)			P_{A-B}
	baseline	one-year follow-up	*P*	baseline	one-year follow-up	*P*	
mRR, ms	1017 ±66.1	909.8 ±104.9	0.03	812.4 ±72.3	920.4 ±164.3	0.02	0.746
SDNN, ms	49.1 ±0.9	38.1 ±9.1	0.59	28.7 ±4.9	54.6 ±8.2	0.02	0.000
SDNNi, ms	47.1 ±1.6	38.2 ±9.04	0.04	778.5 ±224.8	44.2 ±7.9	0.02	0.009
TP, ms^2	2191 ±152	1511.9 ±717.5	0.06	1142.3 ±218.5	2003.4 ±704.9	0.02	0.009
VLF, ms^2	1188.5 ±87.5	763.8 ±368	0.04	569.3 ±221.5	1118.4 ±608.2	0.04	0.004
LF, ms^2	284 ±82	245.6 ±135.5	0.74	264.1 ±88.5	853.8 ±99.4	0.03	0.000
HF, ms^2	120 ±63	120.5 ±77	0.99	995.8 ±673.6	1227.2 ±674.6	0.24	0.000
LF/HF	2.8 ±0.8	2.3 ±0.7	0.66	2.2 ±1.7	2.9 ±1.4	0.46	0.02

P_{A-B} - between subgroups one year after ODT

In particular, a greater increase in SDNN can be noted in Subgroup B (54.6±8.2 ms) than in Subgroup A (38.1±9.1 ms) (*P*=0.000). The VLF power was 1118.4±608.2 ms^2 in Subgroup B and 763.8±368 ms^2 in Subgroup A (*P*=0.004). In Subgroup A, we found no positive dynamics in HRV;

a decrease in mRR from 1017±66.1 ms to 909.8±104.9 ms (P=0.03), SDNNi from 47.1±1.6 ms to 38.2±9.04 ms (P=0.04), as well as VLF power from 1188.5±87.5 ms^2 to 763.8±368 ms^2 (P=0.04). On the contrary, in Subgroup B, a significant positive dynamic of both time-domain and frequency-domain HRV indicators was recorded. SDNN and mRR significantly increased, and not only relative to the initial data, but also more than 50 ms, which significantly reduces the SD risk.[15] The frequency-domain HRV parameters also improved: TP from 1142.3±218.5 ms^2 to 2003.4±704.9 ms^2 (P=0.02), VLF power from 569.3±221.5 ms^2 to 1118.4±608.2 ms^2 (P=0.04), LF power from 264.1±88.5 ms^2 to 853.8±99.4 ms^2 (P=0.03)

Discussion

HRV assessment is well known as a method for predicting unfavorable outcomes in various cardiovascular diseases. The first major study to demonstrate the predictive ability of HRV in relation to overall mortality was the study by Kleiger et al.,[3] which started in 1987 and overturned the accepted ideas about the features of the course of the early period after acute myocardial infarction. In that study, the Holter tapes of 808 patients who survived acute myocardial infarction were analyzed. HRV was defined as SDNN in a 24-hour continuous electrocardiogram recording made 11±3 days after acute myocardial infarction. The mean follow-up time was 31 months. The relative risk of mortality was 5.3 times higher in the group with SDNN less than 50 ms than in the group with SDNN of more than 100 ms. A hypothesis to explain this finding is that decreased HRV correlates with increased sympathetic or decreased vagal tone, which may predispose one to ventricular fibrillation.

According to our study, the dynamics of HRV indices has a significant prognostic value in elderly patients with multivessel coronary artery disease, especially in patients who underwent PCI with stenting of one or more arteries on the background of ODT, and in patients receiving only ODT. In patients who had CABG, the dynamics of HRV indices was not so pronounced, which, most likely, is associated with surgical intervention that disturbs the neurohumoral relations and a relatively short follow-up period.[14] Initially, there were no differences in subgroups, while it should be noted that SDNN values <50 ms were recorded in all 3 groups, which is a marker of a poor prognosis (in particular, ventricular arrhythmias and the SD risk).[16-18]

According to the results of the annual observation, significant differences in the time-domain indicators of HRV between subgroups are recorded. In particular, a significant increase in SDNN can be noted in Subgroup B of patients who underwent PCI. Frequency-domain HRV indices were also significantly better in the subgroup of patients who did not reach any endpoint than in the subgroup of patients who reached at least one endpoint, which indicates an increase in the sympathetic tone of the ANS.

In the ODT group, a similar dynamic was observed: a greater increase in SDNN in Subgroup B than in Subgroup A. Among the frequency-domain HRV indicators, the VLF was a noteworthy index.

Thus, the study of time-domain and frequency-domain HRV indicators is a very promising tool in the study of the ANS in elderly patients with multivessel coronary artery disease. It should be noted that the method has advantages, such as non-invasiveness, safety, and the possibility of long-term observation. The relationship between HRV and the structural and functional state of the heart in CAD, in particular, after CABG, has not been sufficiently studied. In this regard, further research is needed to study HRV in elderly patients.

Conclusions

1. A decrease in HRV is an independent predictor of complications associated with an increase in coronary insufficiency in CAD patients.

2. Such indicators of HRV as SDNN, SDNNi, TP, VLF, and LF have a significant positive predictive value in patients undergoing ODT and/or undergoing PCI.

3. For patients undergoing CABG, at least in the first year after surgery, HRV cannot be considered as an independent prognostic marker.

4. For elderly patients with multivessel coronary artery disease, 24-hour Holter ECG monitoring with subsequent analysis of HRV is recommended to assess the recovery process and pharmacotherapy.

Competing Interests

The authors declare that they have no competing interests.

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The Assessment of Risk Factors for Development of Disability in Children with Congenital Hypothyroidism in Uzbekistan within a Neonatal Screening

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Abstract

The aim of this study was to detect the most significant risk factors leading to disability in children with congenital hypothyroidism (CH) in the autonomous Republic of Karakalpakstan (RK) during neonatal screening (NS).

Methods and Results: We used data of patients with CH registered within NS in the RK in 1998-2019 by the Center for Screening of Mother and Child. To predict and calculate the most significant risk factors for disability in children with CH, we used the method of normalizing intensive indicators by E. Shigan, based on the Bayes theorem.

The study recruited 111 patients with CH aged from 2 months to 20 years. Among the patients, there were 79(71.2%) girls and 32(28.8%) boys. Additionally, 34(30.6%) children with CH had been disabled since childhood. The lack of compensation after the start of treatment had the highest and most significant degree of disability risk (RR=6.39, 95% CI: 7.4-1.2). Among patients diagnosed outside of screening, disability developed 4.1 times more often than with the results of NS (RR=4.0, 95% CI: 1.1-10.6). In CH patients diagnosed outside of screening, "absence of reagents" was a significant factor increasing the risk of disability by 6.1 times (RR=6.1, 95% CI: 1.8-11.2). Such risk factors as "home delivery" and "parental refusal of the primary test" increased the risk of disability by 3.4 times (RR=3.4, 95% CI: 2.5-8.4) and 1.6 times (RR=2.4, 95% CI: 2.93-7.12), respectively. The possible errors or false-negative answers in the "normal" secondary test and the "normal" primary test increased the risk of disability by 3.3 times (RR=4.0, 95% CI: 3.2-10.7) and 2.4 times (RR=2.42, 95% CI: 2.93-7.12), respectively. Factors such as the "late response to retesting" (RR=0.82 95% CI: 0.65-0.54), "late awareness on the part of the medical staff" (RR=0.29, 95% CI: 0.27- 0.08), and "parental refusal of treatment" (RR=1.03, 95% CI: 0.81-0.84) showed less significance in patients' disability. The "starting treatment after 1 month" factor was 4.2 times more likely to result in disability than "starting treatment before 1 month" (RR=4.2, 95% CI: 4.5 -1.1). Cancellation of levothyroxine by parents for children up to 3 years of age and cancellation of treatment by parents after 3 years more likely resulted in disability by 1.4 times (RR=1.43, 95% CI: 1.4 -2.01) and 3.3 times (RR=3.33, 95% CI: 3.3-10.9), respectively.

***Conclusion*:** the most significant risk factors for the development of disability in children with CH in the RK were (in descending order): no compensation after starting treatment, no reagents for screening, starting treatment after 1 year, diagnostics outside of screening, cancellation of L-T4 by parents before and after age 3 years, false-negative secondary TSH test, false-negative primary test, parents refusing the primary test, and childbirth at home.(**International Journal of Biomedicine. 2021;11(3):265-270.**)

Key Words: risk factors • congenital hypothyroidism • disability • neonatal screening

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Abbreviations

CH, congenital hypothyroidism; **CH-C**, congenital central hypothyroidism; **NII**, normalized intensive indicator; **NS**, neonatal screening; **THs**, thyroid hormones; **TSH**, thyroid stimulating hormone.

Introduction

Primary congenital hypothyroidism (CH) is a congenital deficiency of thyroid hormones (THs) in newborns, resulting from incomplete development of the thyroid gland in ontogenesis or from disorder of synthesis and secretion of thyroid hormones, which leads to a lag in the development of all organs and systems of the body, and especially to severe mental retardation and delayed physical development.[1-4] THs are essential for normal growth and development of the central nervous system, especially in the first 3 years of life.[5] During the period of rapid growth and active neurogenesis, the brain becomes especially sensitive to deficiency of thyroxine. Therefore, thyroid failure delays the development of the brain and its maturation, leading to irreversible mental retardation.[1]

The prevalence of CH varies significantly in different countries of the world and ranges from 1 to 2000 to 1 to 3000 newborns. The incidence of CH varies significantly among different ethnic groups and geographic locations, depends on the severity of iodine deficiency in the country, and occurs 2-2.5 times more often in girls than in boys.[1,6-8]

In the mid-70s, many developed countries introduced the state system of NS for CH because of a high incidence of CH, as well as serious consequences and late diagnosis. (9,10) Mass screening was first carried out in Canada in 1974, and today this method is used in the majority of developed countries.[11]

The organization of NS for CH in Uzbekistan is carried out in accordance with the Resolution of the Cabinet of Ministers of the Republic of Uzbekistan, dated 01.04.1998, "On the creation of the State system for early detection of congenital and other pathologies in newborns and pregnant women to prevent the birth of disabled people." A modern screening center was organized in the autonomous Republic of Karakalpakstan (RK) in 1997.[12] Modern screening centers are organized in each region. Diagnostics, treatment, and monitoring of patients with CH aged up to 18 years is carried out free of charge under the supervision of screening centers.

Among the studied risk factors affecting mental and physical development, the most important are: 1) the first therapeutic dose, 2) the age treatment is initiated, 3) the age thyroxine levels are normalized.[13] The latest Guidelines of the European Society for Pediatric Endocrinilogy 2020-2021 confirm that after a newborn receives a positive result of screening for CH, immediate administration of levothyroxine in the first 2 weeks at a dose of 10-15 μg/kg per day is the most optimal.[8]

Despite the development of more accurate test programs, approximately 5% of cases of CH can still be missed in any screening program. Causes may be loss of sample collection, poor samples, misinterpretation of results, subclinical hypothyroidism, or, if TSH is measured alone, failure to detect infants with CH-C.[14]

Any screening system turns into large financial costs for the state. For this reason, any screening system requires periodic evaluation of its effectiveness. In the RK, among 111 patients with CH, 34 are disabled. Therefore, it became necessary to assess the effectiveness of NS in the RK. There were no similar studies in Uzbekistan.

The aim of this study was to detect the most significant risk factors leading to disability in children with CH in the RK during NS.

Materials and Methods

We used data of patients with CH registered within NS in the RK in 1998-2019 by the Center for Screening of Mother and Child. The results of NS for CH were evaluated according to the level of TSH in capillary blood, collected on filter paper from newborns 4-5 days after birth. Testing for THs (TSH, total T4, and total T3) was carried out in the NS laboratory using the DELFIA multifunctional automated immunological laboratory, consisting of a Wallas VICTOR-2D analyzer and a set of auxiliary equipment.

The time for prescribing treatment after birth, the thyroxine dose, and the achievement of the target TSH levels were assessed on the basis of the ESPE recommendations.[3]

To predict and calculate the most significant risk factors for disability in children with CH, we used the method of normalizing intensive indicators by E. Shigan, based on the Bayes theorem.[15]

Based on the literature data, the following risk factors were identified and were included in the analysis: the time of diagnosis during screening, diagnostics outside the screening, the timing treatment was initiated (up to 1 month or after 1 month), compensation after the start of treatment, correctly selected dosage of levothyroxine directly at diagnosis, refusal of treatment by parents, lack of reagents for screening, parents' refusal of primary testing, the rate of "normal" primary test, the rate of "normal" retest, late informing of parents about retesting by medical personnel, late parental response to retesting, childbirth at home, and cancellation of treatment by parents (under age 3 and over age 3).

The NII was calculated for the gradation of each factor, that is, the frequency of cases of disability, according to this gradation, was divided by the total frequency of disability in the surveyed population (30.6%). In order to determine how many times the presence of a factor increases the risk of developing disability, the relative risk index (RR) was calculated. The indicators of RR were also determined according to the gradation of each factor. After determining the NII and RR, for a comprehensive assessment of the phenomenon under study, the corresponding NII values were multiplied by the RR indicators. That is, the integral assessment was determined by the formula: $X = NII \times RR$, where X is the integral risk assessment.

Statistical analysis was performed using Microsoft Excel software package. The normality of distribution of continuous variables was tested by one-sample Kolmogorov-Smirnov test. Baseline characteristics were summarized as frequencies and percentages for categorical variables and as mean±standard deviation (SD) for continuous variables. 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.

Results

The study recruited 111 patients with CH aged from 2 months to 20 years. Among the patients, there were 79(71.2%) girls and 32(28.8%) boys. Additionally, 34(30.6%) children with CH had been disabled since childhood, of which 6(17.6%) had a normal TSH level at Stages 1-2 of screening (the so-called "missed" results), 18(52.9%) children were identified outside of screening, and 10(29.5%) during the screening.

During NS of 87 newborns, disability since childhood was diagnosed in 16(18.4%) of patients. Among 24 children outside NS, disability since childhood was diagnosed in 18(75%). Several factors have been identified that affect the CH diagnosis (Table 1).

Table 1.

Frequency of some risk factors in children with CH and disability

	n	Disability	
		n=34	%
Frequency of disability within NS	87	16	18.4
Frequency of disability outside NS	24	18	75
Risk factors for CH during NS			
	N	n =13	%
Late response to retesting	15	4	26.7
Late awareness on the part of the medical staff	18	2	11.1
"normal" primary test	9	6	66.7
"normal" secondary test	1	1	100
Risk factors for disability and late diagnosis of CH (outside NS)			
	N	n =18	%
Home delivery	2	2	100
Absence of reagents	20	15	75
Parental refusal of the primary test	2	1	50
Risk factors for disability depending on treatment			
	N	n = 8	%
Early onset of treatment (within 1 month)	40	4	10
Compensation after the onset of treatment	18	1	5.6
Decompensation after the onset of treatment	22	3	35.5
Early onset of treatment (within 1 month)			
	N	n =4	%
Cancellation of treatment by parents (before age of 3 years)	7	3	42.9
Cancellation of treatment by parents (after age 3 years)	1	1	100

During the neonatal period, additional risk factors were identified in CH children, affecting treatment and leading to the disability (Table 1). Among patients diagnosed outside of screening, disability developed 4.1 times more often than with the results of NS (RR=4.0, 95% CI: 1.1-10.6). In CH patients diagnosed outside of screening, "absence of reagents" was a significant factor increasing the risk of disability by 6.1 times (RR=6.1, 95% CI: 1.8-11.2). Such risk factors as "home delivery" and "parental refusal of the primary test" increased the risk of disability by 3.4 times (RR=3.4, 95% CI: 2.5-8.4) and 1.6 times (RR=2.4, 95% CI: 2.93-7.12), respectively.

The possible errors or false-negative answers in the "normal" secondary test and the "normal" primary test increased the risk of disability by 3.3 times (RR=4.0, 95% CI: 3.2-10.7) and 2.4 times (RR=2.42, 95% CI: 2.93-7.12), respectively. Factors such as the "late response to retesting" (RR=0.82 95% CI: 0.65-0.54), "late awareness on the part of the medical staff" (RR=0.29, 95% CI: 0.27- 0.08), and "parental refusal of treatment" (RR=1.03, 95% CI: 0.81-0.84) showed less significance in patients' disability (Table 2).

The "starting treatment after 1 month" factor was 4.2 times more likely to result in disability than "starting treatment before 1 month" (RR=4.2, 95% CI: 4.5 -1.1). Cancellation of levothyroxine by parents for children up to 3 years of age and cancellation of treatment by parents after 3 years more likely resulted in disability by 1.4 times (RR=1.43, 95% CI: 1.4 -2.01) and 3.3 times (RR=3.33, 95% CI: 3.3-10.9), respectively. The lack of compensation after the start of treatment had the highest and most significant degree of disability risk (RR=6.39, 95% CI: 7.4-1.2) (Fig.1).

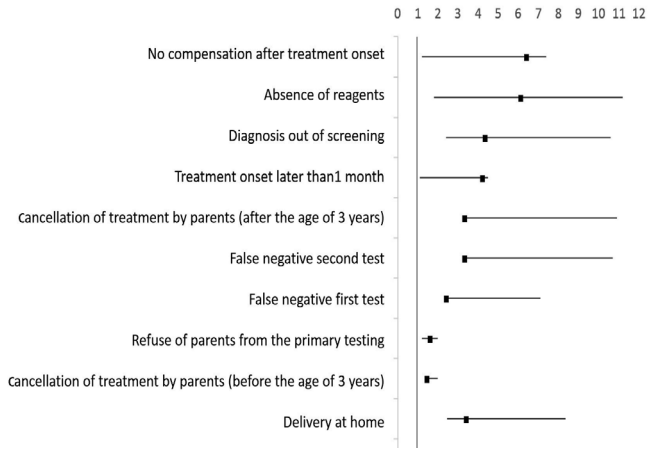

Fig.1. *The most significant risk factors for the development of disability among children with CH*

When assessing thyroid function, out of 40 patients with an early start of treatment with levothyroxine, only 18(45%) children were compensated and 22(55%) patients were decompensated (TSH>5μU/ml). The data obtained confirm that a significant proportion of patients with CH were decompensated (Table 3).

Table 2.

Integral analysis of risk factors for the development of disability among children with CH

Risk factor	Gradation of factors	Disability, %	NII	RR	X
Decompensation after the onset of treatment	No	5.555556	0.1815541	6.4	1.1596036
	Yes	35.483871	1.1596036		7.4065006
Absence of reagents	Yes	75	1.8382353	6.1	11.226366
	No	12.280702	0.3009976		1.8382353
Diagnosis	NS	18.441379	0.5634438	4.0	2.4397115
	Out of NS	75	2.4509804		10.612745
Onset of treatment	Within 1 month	10	0.3267974	4.2	1.0686275
	Later	42.253521	1.380834		4.5153273
Cancellation of treatment by parents (after age 3 years)	Yes	100	3.2679739	3.3	10.893246
	No	30	0.9803922		3.2679739
"normal" secondary test	Yes	100	3.2679739	3.3	10.686275
	No	30	0.9803922		3.2058824
"normal" primary test	Yes	66.666667	2.1786492	2.4	7.124183
	No	27.45098	0.8970909		2.9334871
Parental refusal of the primary test	Yes	50	1.2254902	1.6	1.9980818
	No	30.666667	0.751634		1.2254902
Cancellation of treatment by parents (before age 3 years)	Yes	42.857143	1.4005602	1.4	2.0137087
	No	29.807692	0.9741076		1.4005602
Factors of late onset of treatment (40.8%)					
Home delivery	Yes	100	2.4509804	3.4	8.355615
	No	29.333333	0.7189542		2.4509804
Parental refusal of treatment	Yes	33.333333	0.8169935	1.0	0.8417508
	No	32.352941	0.7929642		0.8169935
Late response to retesting	Yes	26.666667	0.6535948	1.2	0.79064
	No	32.258065	0.7906388		0.95642
No assessment of thyroid function after age 3 years	Yes	25	0.6127451	1.3	0.83241
	Assessed	33.962264	0.8324084		1.13082
No assessment of thyroid function before age 3 years	Yes	14.285714	0.3501401	2.3	0.80532
	Assessed	32.857143	0.8053221		1.85224
No timely ultrasound	Yes	15	0.3676471	2.5	0.90299
	Done	36.842105	0.9029928		2.21788
Late awareness on the part of the medical staff	Yes	11.111111	0.2723312	3.4	0.91392
	No	37.288136	0.9139249		3.06707

Table 3.

TSH level and thyroxine dose depending on the achievement of compensation in children with starting treatment before 1 month

	Compensation after the onset of treatment (n=18)	Decompensation after the onset of treatment (n=22)	*P*-value
TSH before treatment, μU/ml	150.9±44.2	200.5 ±37.5	<0.05
TSH after treatment onset, μU/ml	1.6±0.4	47.9±12.1	<0.05
Levothyroxine dose, μg/day	35 ±3.1	29.3±2.0	>0.05
Levothyroxine dose, μg/kg/day	9.3±0.9	7.3±0.4	<0.05

In patients with congenital hypothyroidism in a state of compensation, the TSH level after the start of treatment was 1.6±0.4 μU/ml vs. 47.9±12.1 μU/ml in decompensation state (P<0.05). In patients with compensation, the prescribed dose of levothyroxine was 9.3±0.9 μg/kg vs. 7.3±0.4 μg/kg in patients with decompensation (P<0.05). It should be noted that in most newborns with congenital hypothyroidism, the prescribed doses of levothyroxine were significantly lower than those recommended by ESPE.[9] In addition, in the first months of life, compensation was assessed based on the total level of T4 only in 22.5% of cases. The lack of compensation after the start of treatment increased the risk of disability in children with congenital hypothyroidism by 6.4 times.

Discussion

In Uzbekistan, according to the results of screening from 1998 to 2017, 5,820,457 newborns were tested, and congenital hypothyroidism was diagnosed in 2323 cases.[16] In the Republic of Karakalpakstan for the period from 2003 to 2019, 383,018 newborns were examined and 124 patients were diagnosed with congenital hypothyroidism. In 2003-2019, the population frequency of congenital hypothyroidism in the Republic of Karakalpakstan, according to the results of neonatal screening, was 1:3089 newborns. Of the children with congenital hypothyroidism, 8(9.9%) were referred to the supervision of an endocrinological dispensary, 1.2%of parents refused treatment, 4(4.9%) moved, and 3 children died (3.7%).[17]

Our study found that girls are more likely than boys to have a congenital hypothyroidism, with a ratio of 2.5:1. According to research by Yang et al.,[18] congenital hypothyroidism was also more prevalent in girls than in boys, but the reason is still unclear.[19]

We identified repeated cases of congenital hypothyroidism in 2 families (3.6%), which coincides with the literature data. According to I.I. Dedov,[1] 85% of congenital hypothyroidism cases are sporadic, and the remaining 15% of cases are caused by dyshormonogenesis.

In the Republic of Karakalpakstan from 2003 to 2019, the annual neonatal screening for congenital hypothyroidismwas an average of 60.6%. The lack of full screening coverage

was mainly due to irregular laboratory testing. For example, due to the lack of reagents, screening was not carried out in 2004 for 1 year. During this period of time, the pediatric endocrinologists and neuropathologists annually diagnosed 2-3 cases of congenital hypothyroidism based on the typical symptoms of the disease. The absence of reagents increased the risk of patients with congenital hypothyroidism developing a disability by 6.1 times, which is consistent with a study by Alimova et al.[20]

Thus, within neonatal screening, 78 patients were diagnosed with congenital hypothyroidism, and in 9 patients the diagnosis of congenital hypothyroidism was not confirmed in Stage 1, due to the low TSH levels, but the clinical signs appeared later together with high TSH levels. Thus, during the screening, 10.3% of children were missed; this is somewhat higher than in a study by A. Büyükgebiz,[14] who found that 5% of children with congenital hypothyroidism might still be missed in any screening program.

During the screening, it was revealed that the time needed to make the diagnosis and prescribe treatment in 78 newborns with congenital hypothyroidism was 1.5 months, on average 46.2±12.4 days after birth with fluctuations from 10 to 207 days, although according to the ESPE recommendations, testing should be done within 2 weeks.[9] This discrepancy is explained by the large distances in the RK between populated areas, cities, and regional centers, so it takes more time to deliver blood samples from maternity hospitals to the screening center. It is proposed to solve these problems by allocating financing to maternity hospitals for postage. Similar problems were identified in a study by Kasatkina et al.[16]

Multivariate analysis confirmed that neonatal screening for congenital hypothyroidism is effective. Outside of screening, disability for congenital hypothyroidism was 4 times higher than during screening. It is necessary to strive for an early start of treatment in the first month of life and use the dose of levothyroxine according to the level of free or total T4 until TSH normalizes. The prescribed first doses of levothyroxine should correspond to international recommendations - 10-15 μg/kg of body weight.

Thus, the most significant risk factors for the development of disability in children with congenital hypothyroidism in the Republic of Karakalpakstan were (in descending order): no compensation after starting treatment, no reagents for screening, starting treatment after 1 year, diagnostics outside of screening, cancellation of L-T4 by parents before and after age 3 years, false-negative secondary TSH test, false-negative primary test, parents refusing the primary test, and childbirth at home.

Competing Interests

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Ophthalmology

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The Effectiveness of Local Conservative Therapy after Panretinal Laser Coagulation against the Background of Diabetic Neuropathy

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Abstract

Background: Panretinal photocoagulation (PRP) (also called scatter laser treatment) remains one of the effective methods of treatment, and it can help to prevent blindness and low vision in diabetic retinopathy (DR). ***The aim*** of this study was to investigate the efficacy of local prolonged conservative therapy after PRP and the effect of somatic polymorbidity on visual functions in patients with diabetic neuropathy (DN) at the stage of clinical manifestations based on monitoring the clinical, functional and morphometric parameters of the macular region of the retina.

***Methods and Results*:** The study included 78 patients with type 2 diabetes (T2D) who underwent PRP for DR using a VISULAS® 532s solid-state laser (ZEISS). The patients were divided into two groups depending on the presence or absence of DN. Group 1 (n=60, 120 eyes) included patients with DN (stage of clinical manifestations), Group 2 (n=18, 36 eyes) included patients without DN. All patients underwent standard ophthalmological examination: visometry, tonometry, perimetry, biomicroscopy of the anterior segment of the eye and vitreous body, and fundus ophthalmoscopy. Thickness map of the retina was obtained using the RTVue-100 OCT (Optovue, Fremont, CA) EMM5 scan protocol and the Stratus OCT (Carl Zeiss Meditec, USA) radial scan protocol. After laser treatment, all patients, regardless of the treatment stage, were prescribed topically Broxinac® (Bromfenac ophthalmic solution 0.09%), 1 drop twice a day for a month. In the presence of macular edema, a carbonic anhydrase inhibitor (Dorzolamide 2% solution) was added to the Broxinac® solution (1 drop twice a day) for up to 1 month after each PRP stage. The dynamics of the parameters of corrected visual acuity and the retinal thickness of the macular region were assessed before PRP and 3 months after the complex treatment.

In patients of Group 1, the dynamics of visual functions against the background of combined treatment (laser and drug) depended on the stage of CKD. Based on the monitoring of the clinical, functional and morphometric parameters of the macular region of the retina after PRP in T2D patients, we found a local, prolonged, 3-month conservative therapy to be effective, using the instillation of Broxinac® supplemented with Dorzolamide 2% solution in the presence of macular edema. Conversely, there is a negative effect of somatic polymorbidity (stage 3 chronic kidney disease), aspartate aminotransferase >40 U/L) on corrected visual acuity and morphometric parameters of the macular region of the retina during PRP in T2D patients with DN at the stage of clinical manifestations.(**International Journal of Biomedicine. 2021;11(3):271-274.**)

Key Words: diabetes mellitus • diabetic neuropathy • panretinal photocoagulation

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Abbreviations

AH, arterial hypertension; **AST**, aspartate aminotransferase; **BMI**, body mass index; **CKD**, chronic kidney disease; **CHD**, coronary heart disease; **CHF**, chronic heart failure; **CVA**, corrected visual acuity; **CVD**, cerebrovascular disease; **DM**, diabetes mellitus; **DN**, diabetic neuropathy; **DR**, diabetic retinopathy; **GFR**, glomerular filtration rate; **HDL-C**, high-density lipoprotein cholesterol; **LDL-C**, low-density lipoprotein cholesterol; **OCT**, optical coherence tomography; **OS**, oculus sinister; **OD**, oculus dexter; **PRP**, panretinal photocoagulation; **TG**, triglycerides; **T2D**, type 2 diabetes.

Introduction

Diabetes mellitus (DM) is one of the most serious chronic diseases that causes a wide range of complications.[1,2] The persistent hyperglycemia can lead to complications affecting eyes, kidneys, nerves, and heart.[2-4] DM is a strong predictor of cardiovascular morbidity and mortality and is associated with both micro- and macrovascular complications. A history of concomitant pathology (arterial hypertension, impaired blood lipid spectrum, chronic heart failure) aggravates the formation of microangiopathies.[4,5] According to various authors, in economically developed countries, the frequency of polyneuropathy in diabetes is from 10% to 90% of observations [6] and directly depends on the duration of the disease.[7] At the initial diagnosis, DN is detected in 12% of patients, and after 20 years - in 50% of patients.[6] Clinical manifestations of DN do not depend on the type of DM.[7,8] With a duration of diabetes for more than 15 years, detectable retinal damage (retinopathy) in 10% of cases leads to low vision and in 2% - blindness.[9]

Panretinal photocoagulation (PRP) (also called scatter laser treatment) remains one of the effective methods of treatment, and it can help to prevent blindness and low vision in DR.[5,10,11] Medical management in patients after PRP usually includes instillation of non-steroidal anti-inflammatory drugs, and in the presence of macular edema, the use of carbonic anhydrase inhibitors.[10,12] According to modern scientific literature, the duration of taking these drugs is usually limited to one or two weeks.[5,9] Considering that PRP is carried out in the presence of pronounced phenomena of retinal vascular endotheliosis (pre- and proliferative processes), a local drug support should be long-term, during all stages of laser coagulation, followed by prolongation until there is an effective decrease in the severity of macular edema and an increase in CVA.

The aim of this study was to investigate the efficacy of local prolonged conservative therapy after PRP and the effect of somatic polymorbidity on visual functions in patients with DN at the stage of clinical manifestations based on monitoring the clinical, functional and morphometric parameters of the macular region of the retina.

Materials and Methods

The study included 78 patients (50 women and 20 men) with T2D who underwent PRP for DR using a VISULAS® 532s solid-state laser (ZEISS). In this study, the DR classification proposed by Kohner and Porta (1992) was used.

Non-proliferative DR was detected in 8(10.2%)[OD]/10(12.8%)[OS] cases, pre-proliferative DR in 48(61.5%)[OD]/42(53.8%)[OS] cases, and proliferative DR in 22(28.2%)[OD]/26(33.3%)[OS] cases, respectively. The exclusion criteria were the presence of the inflammatory, post-traumatic, and dystrophic diseases of the eyeball not associated with DM, as well as hereditary and congenital eye pathologies. PRP was carried out according to the standard method, gradually, in three stages; the interval between the stages of laser treatment was 1 month (29+5 days). The median age of the patients was 64[50;77] years.

The patients were divided into two groups depending on the presence or absence of DN. Group 1 (n=60, 120 eyes) included patients with DN (stage of clinical manifestations), Group 2 (n=18, 36 eyes) included patients without DN. The groups did not differ in gender composition, age, disease duration, or body mass index.

The study was approved by local ethics committee, and written informed consent was obtained from all participants. All patients underwent comprehensive clinical examination. Laboratory methods included a general blood test, general urine analysis, and the assessment of blood levels of creatinine, TG, HDL-C and LDL-C. BMI was calculated using Quetelet's formula (in kg/cm^2). All patients underwent ECG and echocardiography.

All patients were examined by a neurologist, therapist, endocrinologist, cardiologist, and podiatrist. DN was diagnosed by a neurologist based on the assessment of various types of sensitivity (pain, tactile, vibration, cold, and heat) using needles, tuning fork, combined scales of various types of sensitivity, and reflexes. All patients underwent neuromyography using Synapsis, a two-channel electroneuromyographic analyzer. The presence of such concomitant diseases as obesity, AH, dyslipidemia and CHF was taken into account. CKD was diagnosed with determination of the blood creatinine level and further calculation of the GFR using the Cockcroft&Gault formula.

All patients underwent standard ophthalmological examination: visometry, tonometry using non-contact pneumotonometer (Reichert Technologies), perimetry using PNR-2-01, biomicroscopy of the anterior segment of the eye and vitreous body on an SL-140 slit lamp (Carl Zeiss Meditec AG, Germany), and fundus ophthalmoscopy using a non-contact Ocular MaxField High Mag 78D Lens.

Thickness map of the retina was obtained using the RTVue-100 OCT (Optovue, Fremont, CA) EMM5 scan protocol and the Stratus OCT (Carl Zeiss Meditec, USA) radial scan protocol.

After laser treatment, all patients, regardless of the treatment stage, were prescribed topically Broxinac® (Bromfenac ophthalmic solution 0.09%), 1 drop twice a day for a month. In the presence of macular edema, a carbonic anhydrase inhibitor (Dorzolamide 2% solution) was added to the Broxinac® solution (1 drop twice a day) for up to 1 month after each PRP stage. The dynamics of CVA parameters and the retinal thickness of the macular region were assessed before PRP and 3 months after the complex treatment.

Statistical analysis was performed using IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp. The normality of distribution of continuous variables was tested by one-sample Kolmogorov-Smirnov test. Continuous variables with normal distribution were presented as mean (standard error of the mean [SEM]); non-normal variables were reported as median (interquartile range (IQR; 25th to 75th percentiles). Student's unpaired and paired t-tests were used to compare two groups for data with normal distribution. Mann-Whitney U test was used to compare means of 2 groups of variables not normally distributed. The Wilcoxon criterion was used to compare the differences

between the paired samples. The frequencies of categorical variables were compared using Pearson's chi-squared test or Fisher's exact test, when appropriate. A value of $P<0.05$ was considered significant.

Results

Analysis of the presence of concomitant diseases showed a significant predominance of CHD (P=0.037) in patients of Group 1 (Table 1). Before treatment, the CVA values between the groups did not differ. After the combined treatment, there was a tendency to an increase in CVA in both groups. Before PRP, there was a weak correlation with the patient age (r=0.278, P<0.037) in Group 1 and a weak one after PRP (r=0.328, P<0.011) in Group 2. In patients of Group 1, the dynamics of visual functions against the background of combined treatment (laser and drug) depended on the stage of CKD (Table 2). The results of our study revealed a weak correlation between CVA and GFR before and after PRP (r=0.260, P<0.04 and r=0.364, P<0.04, respectively) in patients of Group 1 and a moderate correlation (r=0.412, P<0.001 / r=0.492, P<0.001, respectively) in patients of Group 2. In addition, there was a weak correlation between CVA and AST in both groups (r=0.263, P<0.042) before PRP.

Table 1.

Concomitant diseases in groups

Disease	Group 1 (n=60)	Group 2 (n=18)	*P*-value
CHD, n (%)	30 (50)	4 (22.2)	0.037
CVD, n (%)	10 (16.7)	3 (16.7)	>0.05
Obesity, n (%)	36 (60.0)	10 (55.6)	>0.05
AH, n (%)	52 (86.7)	17 (94.4)	>0.05
CHF, n (%)	41 (68.3)	11 (61.1)	>0.05

Table 2.

The level of CVA depending on the stage of CKD

GFR, ml/min/1.73m^2	Group 1			Group 2		
	n	Before PRP	After PRP	n	Before PRP	After PRP
GFR >90 Stage 1	46	0.51±0.48	0.73±0.27*	20	0.57±0.33	0.66±0.34*
GFR 60 – 89 Stage 2	40	0.51±0.39	0.68 ±0.21	14	0.59±0.25	0.67±0.28*
GFR 30 – 59 Stage 3A/3B	34	0.54±0.17	0.54±0.15	2	0.57±0.02	0.63±0.01

*- $P<0.05$

Of greatest interest are the changes in the morphometric parameters of the macular region of the retina after PRP (Table 3). In general, patients in Group 1 showed a tendency towards a decrease in the retinal thickness in all segments. There was a complete regression of macular edema in 15.0% of cases and a tendency to decrease it in 28.3% of cases, more pronounced in the central and temporal segments. Patients of Group 2 showed a significant (relative to normal values, P<0.05)) decrease in the retinal thickness in the central, superior and inferior segments. In addition, we observed a complete regression of macular edema in 3 patients of this group. The absence of any dynamics in macular edema was found in 10.0% of patients in Group 1 and deterioration of the morphometric parameters of the macular region of the retina in 20.0% of patients with CKD Stage 3A/3B and increased AST level (>40 U/l)..

Domestic and foreign scientists have demonstrated the effect of comorbidity on the development of DN and DR.[4,7] Our study showed that diabetic macroangiopathies, concomitant diseases (arterial hypertension, chronic heart failure), occur with almost the same frequency in both groups, without significantly affecting the development of DN at the stage of clinical manifestations. CHD is the trigger causes for the development of DN at the stage of clinical manifestations.

Table 3.

The retinal thickness (μm) before and after PRP

Segment	Group 1		Group 2	
	Before PRP	After PRP	Before PRP	After PRP
Central OD OS	230.95±69.12 230.10±69.54	220.81±64.25 220.20±81.14	213.0±84.0 210.83±43.28	207.34+11.21* 203.89±12.11*
Nasal OD OS	266.83±29.89 299.90±16.94	267.01±28.16 262.46±33.11	265.33±30.0 265.77±25.35	264.0±29.16 258.27±25.62
Superior OD OS	295.91±157.01 294.51±154.12	286.76±145.23 285.06±105.98	276.05±86.51 277.33±81.25	260.66±29.23* 266.83±23.05*
Temporal OD OS	296.79±117.21 298.65±125.45	288.06±93.17 286.16±99.23	271.22±47.58 267.83±57.45	271.27±44.23 263.83±44.52
Inferior OD OS	286.22±131.14 283.95±111.54	279.65±111.65 276.43±87.56	274.72±102.56 268.95±26.85	265.95±32.56 263.72±27.56*

*- $P<0.05$

We revealed that the CVA level after PRP against the background of long-term local treatment in T2D patients with DN at the stage of clinical manifestations depends on age and the functional state of the liver and kidneys. The combined treatment of DR is not effective in the presence of macular edema, CKD 3A/3B, or changes in the reference parameters of AST and GFR. These criteria are biological markers of a poor prognosis for CVA after PRP and prolonged local conservative treatment. The results obtained require additional research and development of an algorithm for preliminary correction of somatic status in order to increase the effectiveness of PRP in preventing blindness and low vision in this category of patients. Prolonged administration of Broxinac® in Group 2 patients without DN and macular edema is effective in relieving possible complications after PRP. In cases where macular edema is detected in patients without DN, it is also advisable to prescribe Dorzolamide 2%.

In conclusion, based on the monitoring of the clinical, functional and morphometric parameters of the macular region of the retina after PRP in T2D patients, we found a local, prolonged, 3-month conservative therapy to be effective, using the instillation of Broxinac® supplemented with Dorzolamide 2% solution in the presence of macular edema. Conversely, there is a negative effect of somatic polymorbidity (CKD Stage 3**,** AST>40U/L) on CVA and morphometric parameters of the macular region of the retina during PRP in T2D patients with DN at the stage of clinical manifestations.

Competing Interests

The authors declare that they have no competing interests.

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Dermatology

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Polymorphisms in Genes Involved in Steroidogenesis in the Development of Severe Acne

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Abstract

Background: Acne is a multifactorial disease, in the pathogenesis of which one of the leading factors is the excessive effect of androgens on the hair follicles and sebaceous glands, along with hypersecretion of sebum, pathological follicular hyperkeratosis and an inflammatory response. The search for genotypic markers in patients with varying severity of acne is a difficult task due to the multifactorial pathogenesis and the role of trigger factors in the formation of acne. ***The aim*** of this study was to determine SNPs within 3 genes involved in steroidogenesis (*MVK*, *ARPC1B*, and *CA2*) in patients with severe acne.

Methods and Results: The study included 70 patients (42 men and 28 women) aged between 15 and 46 years (the median age - 22.1 years). The main group included 50 patients (29 men and 21 women) with severe acne. The control group consisted of 20 apparently healthy individuals (13 men and 7 women). Molecular-genetic diagnostics was carried out by the method of high-throughput DNA sequencing (next-generation sequencing). Our study showed that severe acne is associated with 12 polymorphic loci of the *MVK* gene (4 SNPs in exons and 8 SNPs in introns), 7 SNPs of the *ARPC1B* gene (2 SNPs in exons and 5 SNPs in introns), and 9 SNPs of the *CA2* gene (3 SNPs in exons and 6 SNPs in introns).

Conclusion: The revealed features of the SNPs within the *MVK*, *ARPC1B*, and *CA2* genes in patients with severe acne probably indicate a hereditary determination of steroidogenesis in acne. The imbalance in the work of the regulatory genes of androgens and estrogens apparently causes an imbalance in the androgen/estrogen ratio in the blood serum towards an increase in androgens against the background of a decrease in estrogens, while their values remain in the normal range. (**International Journal of Biomedicine. 2021;11(3):275-280.**)

Key Words: acne • hair follicle • sebaceous gland • steroidogenesis • single nucleotide polymorphism

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Abbreviations

5α-DHT, 5α-dihydrotestosterone; **17β-HSD**, 17β-hydroxysteroid dehydrogenase; **ARPC1B**, actin related protein 2/3 complex subunit 1B; **CA2**, carbonic anhydrase 2; **DHEAS**, dehydroepiandrosterone sulfate; **DHT**, dihydrotestosterone; **LPS**, lipopolysaccharide; **MVK**, mevalonate kinase; **SNP**, single nucleotide polymorphism.

Introduction

Acne is a multifactorial disease, in the pathogenesis of which one of the leading factors is the excessive effect of androgens on the hair follicles and sebaceous glands, along with hypersecretion of sebum, pathological follicular hyperkeratosis and an inflammatory response. The role of androgens in the pathophysiology of acne has been confirmed by numerous experimental and clinical observations. It is known that the development of prepubertal acne is due to the influence of freely circulating dehydroepiandrosterone sulfate (DHEAS). A positive correlation has been noted for the formation of acne in children with congenital adrenal hyperplasia or virilizing tumors.[1,2]

There is evidence that hair follicles are influenced by both systemically synthesized androgens and locally secreted

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androgen metabolites, such as testosterone and 5α-DHT. At the same time, in the vast majority of acne patients, serum androgen levels remain within normal values.[3] It has been shown that the absence or rarity of acne in men who underwent premature castration before puberty, or in people with androgen insensitivity syndrome, indicates the role of androgens in the etiology of acne.[4]

There is a positive correlation between systemic/local treatment with androgens or anabolic steroids, and serum androgen levels and acne in women. However, women without hyperandrogenism may develop cystic nodular acne and androgenic alopecia. This may be due to the presence of androgen receptors; testosterone and DHT act through a single nuclear receptor, the androgen receptor.[5,6]

The synthesis and composition of sebum is regulated by internal (e.g., PPAR, LXR, RAR, RXR, endocannabinoids) and external (e.g., androgens, Insulin-like growth factor 1, insulin, leptin) factors.[7]

As known, SF is not only a producer of sebum, but also plays a key role in the local immune system of the skin. Thus, sebocytes under the influence of bacterial flora, in particular *Cutibacterium acne* (*C. acnes*), and endogenous mediators, produce a wide range of pro-inflammatory cytokines and growth factors. *C. acnes* induces the secretion of TNFα and IL-8/CXCL-8, and bacterial LPS further increases the level of IL-1α.[8,9] There is evidence that arachidonic acid is an endogenous mediator of inflammation, which in synergy with elevated intracellular calcium levels induces excessive secretion of IL-6 and IL8.[10]

The regulating effect of androgens on sebaceous glands has been established. This effect is realized through androgen receptors expressed on the sebaceous glands. Androgens stimulate the proliferation of the sebaceous glands, and the activation of lipid synthesis in thesebaceous glands occurs in the presence of coactivators, for example, linoleic acid.[11]At the same time, it has been found that androgens have a more pronounced activating effect on the sebaceous gland sebocytes of the facial skin than those in other areas of the skin.[12]

Recent studies have shown that, in addition to interacting with androgens via receptors, sebaceous glands have a number of other important functions. First, the P450 side chain cleavage factors expressed by sebocytes convert cholesterol into pregnenolone. Second, sebaceous glands are capable of secreting testosterone and converting it to 5α-DHT against the background of simultaneous PPAR stimulation.[13] Third, in differentiated sebocytes (in situ) there is an inverse correlation between the expression of 17β-HSD and PPARγ. And fourth, sebocytes have an inactivating function in relation to testosterone through its conversion to androstenedione, and then to 5α-androstenedione.[7]

In contrast to the prolipogenic effect of androgens, estrogens have the opposite effect, namely, they reduce the proliferation of sebocytes and inhibit the production of sebum.[14] Acne patients also have lower levels of serum estradiol and sex hormone binding globulin, and combined oral contraceptives have been effective in acne patients, supporting the idea that estrogens reduce the function of sebaceous glands.[15,16] However, later studies provide conflicting data, in particular, despite the fact that the expression of α- and β-estrogen receptors in the sebaceous glands has been described, 17β-estradiol and progesterone did not have a noticeable effect on either proliferation or lipid production by SZ95 sebocytes.[17]

The search for genotypic markers in patients with varying severity of acne is a difficult task due to the multifactorial pathogenesis and the role of trigger factors in the formation of acne.[18,19] In patients with severe acne, 2 significant SNPs were identified at the 11p11.2 locus (*DDB2*, rs747650 and rs1060573) and one SNP at the 1q24.2 locus (*SELL*, rs7531806), which regulate androgen metabolism, inflammation and scar formation.[20] There is a hypothesis that nodular cystic acne in women without signs of hirsutism is associated with polymorphism of the CAG-repeats of the androgen receptor gene (*ARG*).[21]

The aim of this study was to determine SNPs within 3 genes involved in steroidogenesis (*MVK, ARPC1B*, and *CA2*) in patients with severe acne.

Materials and Methods

The study included 70 patients (42 men and 28 women) aged between 15 and 46 years (the median age - 22.1 years). The main group (MG) included 50 patients (29 men and 21 women) aged between 15 and 46 years (the median age - 23.2 years) with severe acne. The control group (CG) consisted of 20 apparently healthy individuals (13 men and 7 women) aged between 16 and 40 years (the median age - 19.4 years). MG and CG were comparable in age and sex characteristics.

All patients of MG suffered from a severe form of acne, which was clinically characterized by multiple open and closed comedones, inflammatory deep papules, pustules, nodules merging into conglomerates, atrophic scars, post-inflammatory congestive cyanotic spots with predominant localization on the skin of the face, back, and chest. The skin in the lesions had a greasy appearance; subjective sensations were characterized by mild to moderate pain, aggravated by movement and palpation.

Molecular-genetic diagnostics was carried out by the method of high-throughput DNA sequencing (next-generation sequencing) in the Department of Molecular Genetics at the NMRC PHOI, named after Dmitry Rogachev (Moscow, Russia). Genomic DNA was isolated from whole blood samples of examined patients using the CellSep Advanced Kit (DiaSorin Ireland Ltd., Ireland) according to the manufacturer's instructions.

To assess the population frequencies of the identified variants, we used the data of the international project gnomAD Exomes (ExAC) for exon variants and the gnomAD Genomes database for intron variants. For computer assessment of the pathogenicity of the missense variants we found, the programs for predicting the pathogenicity of amino acid substitutions (SIFT, PolyPhen-2, PROVEAN, UMD Predictor) were used. The MutationTaster, Human Splicing Finder, and NNSplice programs were used for computer prediction of the effect of changes in the splicing sites or areas adjacent to the splicing site.

This study was approved by the Ethics Committee of the PRNRMU of the Ministry of Healthcare of the Russian Federation and complies with Ethical Principles for Medical Research Involving Human Subjects, Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964, and amended by the 59th WMA General Assembly, Seoul, Republic of Korea, October 2008. All patients gave their written informed consent.

*S*tatistical analysis was performed using statistical software package XLSTAT 2019. Odds ratios (ORs) and their 95% confidence intervals (CIs) were calculated to determine associations between the polymorphisms and essential hypertension. Two-tailed *P* values <0.05 were considered statistically significant.

Results

In the main group, we identified 21 SNPs within the *MVK* gene (chromosome 12), 12 SNPs within the *ARPC1B* gene (chromosome 7), and 11 SNPs within the *CA2* gene (chromosome 8).

The detailed analysis for the *MVK* gene showed that 5 SNPs were localized in exons (4 synonymous and 1 nonsynonymous), 15 SNPs were localized in introns, and one SNP - in the 5'UTR zone. Analysis of the *ARPC1B* SNPs showed that 3 SNPs were localized in exons, 8 SNPs in introns, and one SNP in the 3'UTR zone. Analysis of the *CA2* SNPs showed that 3 SNPs were localized in exons and 8 SNPs in introns. Characteristics of SNPs of the study genes in exons and introns in acne patients are presented in Tables 1-6.

As shown in Table 1, the *MVK* rs7957619 SNP is localized in exon 3, 2 SNPs in exon 5 (rs34368092 and rs2287218), and 2 SNPs (rs72648042, rs748947620) in exon 9. The OR results showed that 4 out of 5 polymorphic loci of the *MVK* gene in exons [rs7957619 (OR=1.33), rs34368092 (OR=3.01), rs2287218 (OR=3.08), rs748947620 (OR=1.22)] are associated with the development of severe acne (P<0.05), and SNP rs72648042 appears to have a protective effect in the development of acne (OR=0.08).

Table 1.

Characteristics of SNPs within exons of the MVK gene in acne patients

SNPid	Position Chr (12)	Substitution	Exone number	Type and position of substitution	OR
rs7957619	110013879	nonsynonymous	3	c.G155A	1.33
rs34368092	110019233	synonymous	5	c.G405A	3.01
rs2287218	110019338	synonymous	5	c.C510T	3.08
rs72648042	110032871	synonymous	9	c.C768T	0.08
rs748947620	110032922	synonymous	9	c.C819T	1.22

We identified one polymorphic locus the *MVK* gene for the first time, and it was not previously described in any disease (Table 2). The OR results showed that 8 SNPs of the *MVK* gene in introns [rs3759387 (OR=1.30), rs66616264 (OR=1.30), rs6606734 (OR=1.87), rs3752662 (OR=1.30), rs11615637 (OR=3.08), rs2270375 (OR=1.89), rs35191208 (OR=3.08), rs2270374 (OR=3.08)] are associated with the development of severe acne (P<0.05), whereas 7 SNPs in introns [rs61940512 (OR=0.89), rs104895343 (OR=0.40), rs67886029 (OR=0.62), rs7311653 (OR=0.89), -(.) (OR=0.94), rs72648031 (OR=0.62), rs72648041 (OR=0.08)] and SNP rs67606936 in 3'UTR [rs67606936 (OR=0.05)] are likely to have a protective effect (P<0.05) in the development of severe acne (Table 2).

Table 2.

Characteristics of SNPs within introns and 5'UTR of the MVK gene in acne patients

SNPid	Position Chr (12)	Intron / 5'UTR	Type of substitution	OR
rs3759387	110012467	intron	G>T	1.30
rs66616264	110012510	intron	C>G	1.30
rs61940512	110012766	intron	A>G	0.89
rs104895343	110012882	intron	G >A	0.40
rs6606734	110013639	intron	T<G	1.87
rs3752662	110014055	intron	C>T	1.30
rs67886029	110017759	intron	C>T	0.62
rs7311653	110023672	intron	G >A	0.89
rs11615637	110023689	intron	G >A	3.08
rs2270375	110024541	intron	A>G	1.89
-(.)	110028767	intron	TGGTGGGTGGT> GGGGGGGGGGG	0.94
rs35191208	110029008	intron	C>T	3.08
rs2270374	110029186	intron	G >A	3.08
rs72648031	110029279	intron	C>A	0.62
rs72648041	110032661	intron	T>C	0.08
rs67606936	110011515	5'UTR	A>G	0.05

Two SNPs of the *ARPC1B* gene in exons [rs4765 (OR=1.81) and rs144187782 (OR=2.06)] are associated with severe acne (P<0.05), and the rs1045012 SNP (OR=0.62) probably has a protective effect (P<0.05) against the development of severe acne (Table 3). One of the 8 SNPs of the *ARPC1B* gene in introns was described by us for the first time and was not previously described in any disease (Table 4).

Table 3.

Characteristics of SNPs within exons of the ARPC1B gene in acne patients

SNPid	Position Chr (7)	Substitution	Exone number	Type and position of substitution	OR
rs1045012	98984354	nonsynonymous	3	c.G111C	0.62
rs4765	98984399	synonymous	3	c.C156T	1.81
rs144187782	98992084	nonsynonymous	10	c.C1091T	2.06

Table 4.

Characteristics of SNPs within introns and 5'UTR of the ARPC1B gene in acne patients

SNPid	Position Chr (7)	Intron/ 3'UTR	Type of substitution	OR
rs28377312	98984213	intron	G >A	0.62
rs13236076	98985506	intron	G >C	3.06
rs77167227	98985636	intron	C>T	1.22
-(.)	98988999	intron	C>G	2.06
rs10251282	98990236	intron	T>C	0.62
rs529758052	98991916	intron	C>T	1.22
rs41279845	98991939	intron	C>T	0.40
rs10243678	98991944	intron	C>T	1.15
rs704798	98992253	3'UTR	G>T	1.81

Table 5.

Characteristics of SNPs within exons of the CA2 gene in acne patients

SNPid	Position Chr (8)	Substitution	Exone number	Type and position of substitution	OR
-(.)	86377646	synonymous	2	c.C180G	1.22
rs749549639	86385936	nonsynonymous	3	c.C247T	1.22
rs703	86389403	synonymous	5	c.T259C	1.68

The OR results showed that 5 SNPs of the *ARPC1B* gene in introns [rs13236076 (OR=3.06), rs77167227 (OR=1.22), -(.) (OR=2.06), rs529758052 (OR=1.22), rs10243678 (OR=1.15)] and SNP rs704798 in the 3'UTR (OR=1.81) are associated with the development of severe acne (P<0.05), and 3 SNPs [rs28377312 (OR=0, 62), rs10251282 (OR=0.62), rs41279845 (OR=0.40)] seem to have a protective effect in the development of severe acne.

We diagnosed one of the 3 SNPs of the *CA2* gene in exons for the first time both in patients with severe acne and in general among all diseases (Table 5). All 3 SNPs of the *CA2* gene in exons [-(.) (OR=1.22), rs749549639 (OR=1.22), rs703 (OR=1.68)] are associated with severe acne (P<0.05).

Six SNPs of the *CA2* gene in introns (Table 6) [rs190187220 (OR=1.22), rs372186277 (OR=1.22), rs112597132 (OR=2.91), rs2307075 (OR=1.68), rs11780942 (OR=1.15), and rs6605618 (OR=1.15)] are associated with severe acne.

At the same time, 2 SNPs of the *CA2* gene [rs117718682 (OR=0.13), rs552852909 (OR=0.13)] are likely to have a protective value in the development of acne (P<0.05).

Table 6.

Characteristics of SNPs within introns of the CA2 gene in acne patients

SNPid	Position Chr (7)	Type of substitution	OR
rs190187220	86377731	G>C	1.22
rs117718682	86377810	G>T	0.13
rs372186277	86385870	G >A	1.22
rs112597132	86386697	C>T	2.91
rs552852909	86386699	G>C	0.13
rs2307075	86388228	A>C	1.68
rs11780942	86389195	C>T	1.15
rs6605618	86389586	A>C	1.15

Discussion

Our study showed for the first time that there are differences in the frequencies of SNPs within genes involved in steroidogenesis in patients with severe acne. All 21 SNPs of the *MVK* gene, 12 SNPs of the *ARPC1B* gene, and 11 SNPs of the *CA2* gene were first identified by us in patients with severe acne.

The results obtained on the difference in the frequencies of SNPs of genes encoding steroidogenesis indicate the genetic regulation of both the synthesis of steroid hormones and the response of a complex of specific tissue receptors localized both in the affected skin itself and in steroid-sensitive tissues. This provides a synergistic local and systemic response to steroid hormones and their metabolites with the development of a pathological skin process in acne.

The *MVK* gene encodes the peroxisomal enzyme mevalonate kinase. Mevalonate is a key intermediate

product, and mevalonate kinase is a key early enzyme in the synthesis of isoprenoids and sterols. In addition, expression of the *MVK* gene protects keratinocytes from UFO-induced apoptosis. Mutations of the *MVK* gene are probably responsible for the impaired synthesis of metabolites of steroid hormones, as well as an imbalance in the natural apoptosis of keratinocytes.

The *ARPC1B* gene encodes one of 7 subunits of the human Arp2/3 protein complex. It has been shown that the ARP2/3 complex is co-localized with actin at the immune synapse in CD8+ human cytotoxic T-lymphocytes. In addition to its role in the cytoplasmic cytoskeleton, the Arp2/3 complex also promotes actin polymerization in the nucleus, thereby regulating gene transcription and repair of damaged DNA. It has also been shown that this gene is responsible for the tissue response to estrogens and estradiol. It is likely that the *ARPC1B* SNPs result in an imbalance in the response of tissues, including skin, to estrogens, which contributes to the development of the pathological process in acne.

The *CA2* gene encodes a protein that is one of several isoenzymes of carbonic anhydrase, which are in the family of zinc metalloenzymes. The *CA2* SNPs additionally affect the imbalance in the response of tissue receptors to steroid hormones in acne.

Our study showed that severe acne is associated with 12 polymorphic loci of the *MVK* gene (4 SNPs in exons and 8 SNPs in introns), 7 SNPs of the *ARPC1B* gene (2 SNPs in exons and 5 SNPs in introns), and 9 SNPs of the *CA2* gene (3 SNPs in exons and 6 SNPs in introns).

Conclusion

The revealed features of the SNPs within the *MVK*, *ARPC1B*, and *CA2* genes in patients with severe acne probably indicate a hereditary determination of steroidogenesis in acne. The imbalance in the work of the regulatory genes of androgens and estrogens apparently causes an imbalance in the androgen/estrogen ratio in the blood serum towards an increase in androgens against the background of a decrease in estrogens, while their values remain in the normal range.

Thus, our studies made it possible to identify and characterize the polymorphic loci of the *MVK*, *ARPC1B*, and *CA2* genes involved in the pathophysiological mechanism of the genetic determination of steroidogenesis in acne.

Competing Interests

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

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Spatial Distribution of the N2 and P300 Components of the Auditory Evoked Potential in Women with Arterial Hypertension: A study in the Russian Arctic

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Abstract

The aim of this study was to evaluate the spatial distribution of the latencies and amplitudes of the N2 and P3 (or P300) components of the auditory evoked potential (AEP) in the Russian Arctic working-age women with different levels of blood pressure (BP).

Methods and Results: A total of 25 working-age women living in Nadym city for more than 20 years took part in this study. Group 1 (n=12, control group) consisted of women with BP within the normal range (<130/90 mmHg); Group 2 (n=13) consisted of women with arterial hypertension (AH) (AH duration from 1 to 10 years). The parameters of the N2 and P300) components of the AEP were evaluated using an electroencephalograph (Neuron-Spectrum-4/VPM, Russia). An auditory oddball paradigm was used to elicit the oddball event-related potentials **(**ERPs).

In Group 2, compared with Group 1, the N2 latency was more pronounced in the parietal (P4, P3), central (C4, C3), frontal (F4, F3), and left temporal (T3, F7) regions. The N2 amplitude in all studied brain regions in individuals of both groups was comparable. The P300 latency did not differ between the two groups. In Group 2, the P300 amplitude was significantly lower in the parietal region (P3) on the left, and in the central and temporal regions on the right (C4, T4). In Group 2, inverse correlations between DBP and the P3 amplitude were revealed in the central (C4: r=-0.88, *P*=0.001; C3: r=-0.86, *P*=0.001), frontal (F4: r=-0.76; *P*=0.01; F3: r=-0.93, *P*=0.001), and anterior-temporal cerebral regions (F8: r=-0.65, *P*=0.04; F7: r=-0.64, *P*=0.04). SBP correlated with the P3 amplitude in the right mid-temporal region (T4: r=0.64, *P*=0.04).

Conclusion: The features of the spatial distribution of the N2 and P300 components of the AEP can be used for early diagnosis of the risk of developing cognitive disorders in AH patients.(**International Journal of Biomedicine. 2021;11(3):281-285.**)

Key Words: auditory evoked potential • cognitive impairment • arterial hypertension

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Abbreviations

AH, arterial hypertension; **AEP**, auditory evoked potential; **CI**, cognitive impairment**; BP**, blood pressure; **DBP**, diastolic BP; **SBP**, systolic BP; **ERP**, event-related potential.

Introduction

Arterial hypertension (AH) is a strong independent risk factor for the development of cognitive impairment and dementia.[1,2] The early detection of cognitive impairments[3,4] is critical for effective treatment and prevention of dementia. However, early detection of cognitive impairment (CI) is a serious clinical problem. The uncomfortable climatic conditions of the Arctic increase the risk of developing AH, and in certain cases may contribute to the development of CI. Neuropsychological methods for detecting cognitive disorders are not always recognized as sufficiently reliable.[5,6]

Event-related potentials (ERPs)**,** evoked by the auditory and visual senses, are one of the most commonly used tools

to assess the neural basis of sensory perception and cognitive processing in humans.[7,8] The associated ERP is composed of several components such as N1, P2, N2, and P3 (or P300), indicating early/pre-attentive to late selective attention and cognitive processing.[9] The N2 component may reflect the attended mismatch detection,[10] whereas the P3 component is assumed to be an indicator of controlled processing.[11] A variety of psychological and biological factors may modulate the latency and amplitude of the ERP components. Shorter latencies indicate superior mental performance relative to longer latencies. Reduced P300 amplitude and latency prolongation have been reported in patients with AH, schizophrenia, and Parkinson's disease.[12-15] Cicconetti et al.[16] showed that the N2 latency was significantly higher in patients with isolated systolic hypertension (ISH) vs. controls (P<0.0001), suggesting the existence of early subclinical alterations in neurocognitive function in early ISH.

AH in residents of the Arctic zone of Russia is characterized by a more severe course than in residents of middle latitudes. AH also occurs in young people with early damage to target organs, frequent hypertensive crises and impaired higher nervous activity.[17,18] Women make up the active able-bodied part of the population, but the uncomfortable influence of the Arctic climate contributes to early age-related endocrine disorders,[19] which may also affect the state of vascular tone. Therefore, the improvement of instrumental methods for early diagnosis of the risk of cognitive disorders in the working-age residents of the Arctic zone of the Russian Federation, especially in women, is necessary and relevant.

The aim of this study was to evaluate the spatial distribution of the latencies and amplitudes of the N2 and P300 components of the auditory evoked potential (AEP) in the Russian Arctic working-age women with different levels of BP.

Materials and Methods

A total of 25 working-age women living in Nadym city for more than 20 years took part in this study. Group 1 (n=12, control group) consisted of women with BP within the normal range (<130/90 mm Hg); Group 2 (n=13) consisted of women with AH (AH duration from 1 to 10 years). AH women either did not adhere to regular antihypertensive therapy (n=7), or periodically received monotherapy (n=6) (angiotensin-converting enzyme inhibitors, angiotensin receptor blockers). The average age of persons in Group I was 43(36.5;55.5) years, in Group 2 - 49.0(47.0;53.0) years.

Office BP was measured using a mercury sphygmomanometer, according to Korotkov's method. BP was measured 3 times, and the means of these measurements were used in the analyses.

The parameters of the N2 and P300 components of the AEP were evaluated using an electroencephalograph (Neuron-Spectrum-4/VPM, Russia). An auditory oddball paradigm was used to elicit the oddball ERPs. Binaural nonverbal acoustic stimulation was performed with a stimulus duration of 50 ms, an intensity of 80 dB, a period between stimuli of 1sec, a tone frequency of 2000 Hz (for a target stimulus, probability of 25%) and 1000 Hz (for a non-target stimulus, probability of 75%). The P3 was identified as a positive component wave of the ERP, occurring 250-300 ms after stimulus onset. The N2 was identified as a negative component wave of the ERP, occurring 200ms after presentation of stimulus. The amplitude (mcV) and latency (ms) of the N2 and P3 components of the AEP in the brain regions were estimated. The International System 10–20 of the American Society of Electroencephalography was used for electrode placement. The studied brain regions included F3, F4 (frontal), C3, C4 (central), P3, P4 (parietal), and F7, F8, T3, T4 (temporal) leads.

Statistical analysis was performed using the statistical software «Statistica» (v. 13.0, StatSoft, USA). We evaluated the normal distribution of latency and amplitude measures by the Shapiro-Wilks test. Median values are presented with interquartile (IQ) ranges (IQR; 25th to 75th percentiles). Mann-Whitney U test was used to compare differences between two independent groups. The Spearman correlation coefficient (r_s) was used to assess the relationship between variables. A value of $P<0.05$ was considered significant.

The study was approved by the Ethics Committee of the N. Laverov Federal Center for Integrated Arctic Research, RAS (Protocol №1, 11.16.2017). Written informed consent was obtained from all participants.

Results

The SBP and DBP levels in Group 2 were significantly higher than in Group 1 (138(133;141) mmHg and 91.5(90;98) mmHg versus 116.5(112.5;126) mmHg and 79.5(76;84.5) mmHg, respectively (P<0.05). In Group 2, compared with Group 1, the N2 latency was more pronounced in the parietal (P4, P3), central (C4, C3), frontal (F4, F3), and left temporal (T3, F7) regions (Table 1). The N2 amplitude in all studied brain regions in individuals of both groups was comparable.

Table 1.

Spatial distribution of the N2 latency (ms) in the study groups (Me (P_{25}; P_{75})

EEG lead	Group 1 (n=12)	Group 2 (n=13)	P-value
P4	199.5 (189.5;214.0)	231.0 (214.0;269.0)	0.001
P3	200.5 (192.0;209.0)	233.5 (225.0;247.5)	0.006
C4	206.0 (192.5;217.0)	241.5 (220.0;264.0)	0.006
C3	206.0 (192.0;217.0)	239.0 (231.0;247.0)	0.001
F4	209.0 (200.5;211.5)	244.5 (225.0;269.0)	0.002
F3	209.0 (200.5;214.0)	250.0 (242.0;258.0)	0.001
T4	220.0 (208.5;220.0)	225.0 (203.0;264.0)	0.459
T3	203.0 (195.0;214.5)	233.5 (220.0;258.0)	0.011
F8	211.5 (206.0;217.0)	228.0 (217.0;258.0)	0.054
F7	220.0 (206.0;220.0)	242.4 (225.0;258.0)	0.027

The P300 latency did not differ between the two groups. The range of P300 latency in all brain regions in Groups 1 and 2 was 264-346ms (min-max) and 275-395ms, respectively. In Group 2, the P300 amplitude was significantly lower in the parietal region (P3) on the left, and in the central and temporal regions on the right (C4 T4) (Table 2). In Group 2, the P300 amplitude in the temporal regions was the smallest, at the lower limit of the age standard (>5 mcV).

Table 2.

Spatial distribution of the P300 amplitude (mcV) in the study groups (Me (P_{25}; P_{75})

EEG lead	Group 1 (n=12)	Group 2 (n=13)	*P*-value
P4	13.5(9.7;18.0)	8.7(5.0;10.8)	0.067
P3	13.9(10.2;19.0)	7.7(5.9;12.9)	0.021
C4	17.1(11.3;18.9)	9.4(6.2;15.3)	0.043
C3	14.3(10.9;21.5)	7.9(5.5;14.4)	0.067
F4	14.1(9.9;23.8)	12.1(7.7;16.8)	0.359
F3	11.6(9.7;25.3)	10.4(6.9;14.8)	0.408
T4	10.9(7.7;18.3)	6.9(5.4;11.1)	0.04
T3	12.6(11.1;17.1)	10.9(6.2;12.9)	0.130
F8	8.4(7.5;16.1)	6.9(5.6;9.9)	0.408
F7	8.1(6.4;14.2)	6.4(4.7;8.9)	0.161

In Group 2, inverse correlations between DBP and the P300 amplitude were revealed in the central (C4: r=-0.88, *P*=0.001; C3: r=-0.86, *P*=0.001), frontal (F4: r=-0.76; *P*=0.01; F3: r=-0.93, *P*=0.001), and anterior-temporal cerebral regions (F8: r=-0.65, *P*=0.04; F7: r=-0.64, *P*=0.04). SBP correlated with the P300 amplitude in the right mid-temporal region (T4: r=0.64, *P*=0.04). In Group 1, no significant correlations were found.

Our results showed that in women with AH, the N2 latency was longer in the parietal (P4, P3), central (C4, C3), frontal (F4, F3), and left temporal (T3, F7) regions of the brain, compared with the control group. The N2 component is associated with the processes of primary recognition and differentiation of the stimulus.[6] According to A.R. Luria's theory, higher cortical functions are provided by the integrated activity of the entire brain as a whole. However, different parts of the brain are not equivalent.[20] In particular, Luria notes that in case of damage to the structures of the functional energy block, which includes the subcortical-stem structures of the brain, the response time to external stimuli increases, which leads to a slowdown in all cognitive processes and a decrease in concentration.[20,21] Another functional block includes the secondary and tertiary zones of the cortical analyzers of somatic sensitivity, hearing, and vision, reflected in the bioelectrical activity of the parietal, temporal, and occipital cerebral lobes. Its function is the perception, recognition, and storage of information received from the outside world. When the structures of this block are damaged, the perception of information (gnosis) is impaired.[20] The third block is responsible for programming actions and decision-making with the participation of the frontal cerebral lobes.[21]

Lacunar cerebral infarctions with typical localization (thalamus, subcortical basal ganglia) for AH and diffuse changes in white matter can be the morphofunctional basis of cognitive disorders.[22] Cognitive disorders can also occur against the background of acceleration of degenerative processes in the cerebral cortex.[23] In the case of multiple small-focal lesions of the deep parts of the brain, disorders of higher mental functions are caused by the disconnection of the brain structures, in particular, due to damage to the connections of the frontal regions with the temporal, parietal cerebral regions, as well as the structures of the limbic-reticular complex.[24] Functionally important for cognitive functions are such parts of the brain as the frontal lobes, the temporo-parieto-occipital regions, the mediobasal regions of the temporal lobe, the anterior and middle parts of the visual hillocks associated with the frontal lobes of the brain and the limbic system, the posterior-lower-lateral region and the dentate nucleus of the cerebellar hemisphere, contralateral to the dominant cerebral hemisphere, globus pallidus.[24] All the above morphological and functional changes in the brain are reflected in the spatial distribution of the parameters of all AEP components. Thus, given that the N2 component reflects the contribution of cortical, thalamic, and stem bioelectric generators,[25] it is assumed that stimulus recognition occurs with the participation of the temporal region with simultaneous connection of the associative parietal lobes of the brain.[26] A more pronounced lengthening of N2 latency in the left brain regions in AH persons is possibly associated with a decrease in neuronal activity of central regulators of parasympathetic control of vascular tone, mostly located in the left hemisphere.[27] We believe that in AH patients, the primary recognition and classification of the stimulus occurs more slowly than in people with normal BP.

The late P3 (P300) component is associated with the final identification of the stimulus that requires comparing it with a pattern in memory and making a decision regarding the action associated with it. Of particular importance in its formation are the processes of directed attention and short-term memory.[28] As for the physiological aspect of P300 and its relationship with cortical networks, various studies have shown that several cortical generators of P300 can coexist: the medial temporal lobe, the temporo-parietal junction, and the medial and lateral frontal lobes.[29] The presence of cognitive disorders is characterized by a lengthening of the P300 latency and a decrease in the P300 amplitude.[21] It is believed that the P300 amplitude reflects the relative amount of neuronal resources involved in stimulus processing.[30] The P300 amplitude is also proportional to the amount of attention resources allocated to a specific task and is related to memory performance.[31] In our study, there were no differences in the P300 latency between the AH group and control group, which may indicate the absence of violations of the final identification of the stimulus and decision-making. However,

a decrease in the P3 amplitude, especially in the temporal regions to marginal values (median 6.4-6.9 μV), may indicate a change in the activity of the neurotransmitter system, a decrease in the cognitive resource to ensure decision-making. The positive correlations between SBP and P3 amplitude in the right midtemporal region (r=0.64, *P*=0.04) that we revealed indicate the need to maintain a relatively elevated BP for adequate cerebral perfusion and preservation of cognitive functions. However, further preservation of the elevated SBP leads to the risk of CI.

Launer et al. [2] found an increased risk for intermediate and poor cognitive function with every increment increase in SBP by 10 mmHg irrespective of the history of stroke, coronary heart disease, and subclinical atherosclerosis indicating that BP control in early life may reduce the risk for CI in old age.

In our study, inverse correlations between DBP and the P3 amplitude were found in the central, frontal, and anterior temporal regions of both hemispheres. A number of AH studies[14,15] revealed not only an increase in the P300 latency and a decrease in its amplitude, but also a relationship between changes in P3 to a greater extent with the DBP level. In a study by Lv et al.,[32] U-shaped associations were identified between CI and SBP, DBP. The cut-points at which risk for CI was minimized were determined by quadratic models as 141 mmHg and 85 mmHg, respectively. Below the identified cut-points, each 1mmHg decrease in BP corresponded to 0.7% and 1.1% greater risk in the risk of CI, respectively. Above the cut-points, each 1mmHg increase in BP corresponded to 1.2% and 1.8% greater risk of CI for SBP and DBP. We believe that the decrease in cognitive resources is more influenced by an increase in DBP, especially in the Arctic zone.

In conclusion, in women with hypertension, the N2 latency is longer in the parietal (P4, P3), central (C4, C3), frontal (F4, F3) and left temporal regions (T3, F7), and the P3 amplitude is lower in the right central (C4) and mid-temporal (T3) regions and the left parietal region of the brain, compared to individuals with normal BP. This may indicate a slowdown in brain processes during the initial recognition and differentiation of stimuli and a decrease in neuronal resources in people with high BP already at working age. The absence of significant changes in the P300 latency reflects the preservation of the speed of cognitive processes associated with the final identification of the stimulus and decision-making. The revealed inverse correlations between DBP and the P300 amplitude in the frontal, central, and anterior temporal regions of the brain reflect the relationship between a decrease in cognitive resources and an increase in DBP. The features of the spatial distribution of the N2 and P300 components of the AEP can be used for early diagnosis of the risk of developing cognitive disorders in AH patients.

Competing Interests

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Experimental Medicine

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A Study of the Influence of New Generation Granulated Sorbents on the Processes Regulating the Aggregate State of the Blood with the Use of Piezoelectric Thromboelastography

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Abstract

***Background*:** Gastroduodenal bleeding (GDB) and the improvement of endoscopic hemostasis (EH) remain a priority in emergency surgery. This article presents the results of an experimental study of the effects of granular sorbents (Aseptisorb, Aseptisorb-A, Aseptisorb-D) on the system regulating the aggregate state of the blood using modern capabilities of piezoelectric thromboelastography (TEG).

Methods and Results: The study involved 12 healthy volunteers (9/75% men and 3/25% women) aged between 18 and 58 years, with the average age of 34.0(26.0;44.0) years.

For the study, the blood of healthy volunteers with normal indicators of the system regulating the aggregate state of the blood was used. In vitro experiments: Several tests were performed with the blood of each volunteer. In the first experiment (the control stage), the blood cuvette did not contain the test material. At the second stage of the experiment, the hemostatic properties of new generation granulated sorbents (Aseptisorb, Aseptisorb-A, and Aseptisorb-D) were studied.

Experimental studies have shown that the use of granular sorbents Aseptisorb, Aseptisorb-A, and Aseptisorb-D in varying degrees affects the links of platelet and coagulation hemostasis, providing acceleration of thrombosis processes while increasing the maximum density of the clot. These effects determine the effectiveness of the clinical use of these sorbents to stop various types of bleeding.

Conclusion: Experimental studies of the effect of granular sorbents on the system regulating the aggregate state of the blood using piezoelectric TEG have shown that the use of Aseptisorb, Aseptisorb-A, and Aseptisorb-D can significantly reduce the time of blood clotting and increase the maximum clot density, which determines the possibility of the use of these sorbents in endoscopic hemostatic treatment for GDB. (**International Journal of Biomedicine. 2021;11(3):286-290.**)

Key Words: piezoelectric thromboelastography • the aggregate state of the blood • granular sorbents

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Abbreviations

CPC, contact phase of coagulation; **EH**, endoscopic hemostasis; **GDB**, gastroduodenal bleeding; **TEG**, thromboelastography; **U**, units.

Introduction

The problem of bleeding and the improvement of surgical hemostasis methods has remained a priority in emergency surgery for many decades. One of the most technically complex types of hemostasis is endoscopic arrest of Gastroduodenal bleeding (GDB).[1-6] Modern endoscopy has many ways to stop bleeding, among which the most common are coagulation

methods (argonplasma coagulation, diathermocoagulation, laser photocoagulation, etc.), injection hemostasis, clipping, and application methods, as well as combined techniques. Even so, the rate of recurrence of hemorrhage, even after successful primary endoscopic hemostasis (EH), reaches 10%-46%, which shows the need to improve the capabilities of therapeutic endoscopy.[7-11]

Methods of EH by insufflation of powdered hemostatic systems, such as Hemospray, EndoClot, etc., to the source of hemorrhage are becoming increasingly widespread globally in clinical practice. The main disadvantage of such systems is their high cost, which limits the possibility of their use in everyday clinical practice.[12,13] It should be noted that using powdered hemostatic systems in therapeutic endoscopy is not new. For more than 25 years, granular sorbents with hemostatic, antibacterial, and other properties have been successfully used for endoscopic hemostasis of gastroduodenal bleeding. However, the mechanism of action of these sorbents on the system of regulation of the aggregate state of blood remains not fully understood.[14-17]

The aim of our research was to study the peculiarities of the influence of new generation granular sorbents on the system regulating the aggregate state of the blood through *in vitro* experiments using the modern possibilities of piezoelectric TEG.

Materials and Methods

The study involved 12 healthy volunteers (9/75% men and 3/25% women) aged between 18 and 58 years, with the average age of 34.0(26.0;44.0) years.

For the study, the blood of healthy volunteers with normal indicators of the system regulating the aggregate state of the blood was used. In the aseptic conditions, venous blood was collected with a venipuncture needle into vacuum tubes containing a 3.8% sodium citrate solution with a volume of 4.5 ml, intended for coagulographic studies. The contents of the test tube were carefully mixed by tilting the test tube several times.

The study of the parameters of the regulation of the aggregate state of the blood was performed using the piezoelectric thromboelastograph ARP-01M "Mednord."

In vitro experiments: Several tests were performed with the blood of each volunteer. In the first experiment (the control stage), the blood cuvette did not contain the test material. Next, 0.3 ml of citrate blood was added to the TEG cuvette using a laboratory single-channel pipette dispenser, then the blood cuvette was placed in the device's thermostat chamber and a coagulation activator solution (0.025 M calcium chloride solution) was added. The sensor of the device was immersed in the cuvette and the study was started.

At the second stage of the experiment, the hemostatic properties of new generation granulated sorbents (Aseptisorb, Aseptisorb-A, and Aseptisorb-D) were studied. To do this, 1.0 mg of the sorbent was added to the cuvette of the device filled with 0.3 ml of citrate blood. The powdered sorbent was evenly mixed with the test blood, then the activator solution was added and the study was started.

The results of the studies were analyzed by evaluating the following parameters of thromboelastograms: the time of the CPC, the intensity of the CPC, the time to reach the thrombin constant, the constant of thrombin activity, the time of blood clotting, the intensity of the coagulation drive, the time of clot polymerization, the intensity of clot polymerization, the time of fibrin-platelet clot formation, the maximum density of the clot, the intensity of total clotting, and the time of the beginning of fibrinolysis, according to the device instructions.[18-19]

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 Voronezh State Medical University named after N. N. Burdenko. Written informed consent was obtained from all participants.

Statistical analysis was performed using Microsoft Excel software package. For descriptive analysis, results are presented as median (Me), lower quartile (Q_l) and upper quartile (Q_u). A non-parametric Kruskal-Wallis test was used for comparisons of median values among four groups, followed by post-hoc testing using un-paired Mann-Whitney U tests.

Results and Discussion

When analyzing thromboelastograms of healthy volunteers, it was found that the time of the CPC was 1.0(1.0;1.0)min, while the intensity of the CPC was at the level of 39.5(17.7;63.0)U (Table 1). The time to reach the thrombin constant occurred at 5.9(4.6;7.0)min, and the constant of thrombin activity was at the level of 17.8(13.6;27.4)U. Blood clotting in healthy individuals occurred at 12.1(10.7;14.1)min, with the intensity of the coagulation drive of 19.3(10.8;21.9) U. Clot polymerization occurred at 23.5(20.7;25.3)min, and the intensity of clot polymerization was 13.1(5.3;14.5) U. Formation of the fibrin-platelet clot was observed at 32.6(29.9;38.8) min. The maximum clot density in healthy individuals was 389.0(289.0;444.5)U with the total clotting intensity at 7.9(6.1;11.8)U. At the same time, it should be noted that in 3 observations, the clot lysis process began at 29.7(29.5;29.9)min.

Aseptisorb effects

When studying the effect of Aseptisorb on the dynamics of the processes regulating the aggregate state of the blood, we found no significant changes in the time of the CPC compared to the control stage. The time of the CPC for Aseptisorb was 1.0(1.0;1.0)min, but the intensity of the CPC was at a higher level and amounted to 80.5(70.0;108.5)U (P=0.002). Under the influence of Aseptisorb, the time to reach the thrombin constant occurred earlier than in the control stage (P=0.003). At the same time, the constant of thrombin activity was also more pronounced, 54.1(25.9;62.5)U (P=0.002). The use of Aseptisorb significantly reduced the blood clotting time from 12.1(10.7;14.1)min to 6.8(5.6;8.8)min (P=0.000) and increased the intensity of the coagulation drive from 19.3(10.8;21.9)U to 34.6(16.5;41.3)U (P=0.03). The onset of clot polymerization occurred at an earlier time than in the control stage [(16.8(15.6;18.8)min and 23.5(20.7;25.3) min, respectively, P=0.000)], but the intensity of clot

polymerization did not differ significantly from the control stage. A fibrin-platelet clot was formed at 28.1(24.3;29.7) min versus 32.6(29.9;38.83)min in the control stage (*P*=0.04). Analyzing the characteristics of the clot density, we found that due to the sorbtion activity of Aseptisorb and its hydrophilic properties, the maximum clot density was higher than in the control stage [(468.5(438.0;696.5)U and 389.0(289.0;444.5)U, respectively, *P*=0.005)], as was the intensity of total clotting [(12.3(10.6;19.8)U and 7.9 (6.1;11.8)U, respectively, *P*=0.005)]. Clot lysis in the use of Aseptisorb was not observed in any study.

Aseptisorb-A effects

Analyzing the effect of Aseptisorb-A on the parameters of the regulation of the blood aggregate state, we found that, like Aseptisorb, Aseptisorb-A had no effect on the time of the CPC. The time of the CPC for Aseptisorb was 1.0(1.0;1.0)min. However, the use of Aseptisorb-A allowed an increase in the intensity of the CPC to 99.5(67.0;158.5) U from 39.5(17.3;63.0)U in the control stage (*P*=0.001). For Aseptisorb-A, the time to reach the thrombin constant was 3.3(2.2;4.6)min, and the thrombin activity constant was 58.3(30.8;97.2)U. The use of Aseptisorb-A reduced the blood

Table 1

The influence of new generation granular sorbents on TEG parameters

Indicator	Control stage	Aseptisorb	Aseptisorb-A	Aseptisorb-D	*P*-value
Time of the CPC (min)	1.0(1.0;1.0)	1.0(1.0;1.0)	1.0(1.0;1.0)	1.0(1.0;1.0)	>0.05
P-value	-	>0.05	>0.05	>0.05	-
The intensity of the CPC	39.5(17.7;63.0)	80.5(70.0;108.5)	99.5(67.0;158.5)	117.5(83.0;206.5)	<0.001
P-value	-	0.002	0.001	0.001	-
The time to reach the thrombin constant (min)	5.9(4.6;7.0)	2.8(2,6;4.8)	3.3(2.2;4.6)	2.1(1.6;2.8)	0.001
P-value	-	0.003	0.001	0.000	-
The constant of thrombin activity	17.8(13.6;27.4)	54.1(25.95;62.5)	58.3(30.8;97.2)	71.8(54.4;125.0)	<0.001
P-value	-	0.002	0.000	0.000	-
The time of blood clotting (min)	12.1(10.7;14.1)	6.8(5.6;8.8)	6.1(4.2;7.3)	4.2(2.5;5.4)	0.000
P-value	-	0.000	0.000	0.000	-
The intensity of the coagulation drive	19.3(10.8;21.9)	34.6(16.5;41.3)	34.2(23.3;55.6)	46.7(30.8;70.4)	0.001
P-value	-	0.03	0.003	0.000	-
The time of polymerization of the clot (min)	23.5(20.7;25.3)	16.8(15.6;18.8)	16.1(14.2;17.3)	14.2(12.5;15.4)	0.000
P-value	-	0.000	0.000	0.000	-
The intensity of polymerization of the clot	13.1(5.3;14.5)	11.2(6.6;19.4)	10.7(8.7;16.4)	10.4(7.8;17.3)	>0.05
P-value	-	>0.05	>0.05	>0.05	-
The time of fibrin-platelet clot formation (min)	32.6(29.9;38.8)	28.1(24.3;29.7)	25.8(24.5;28.0)	28.7(26.0;29.5)	0.03
P-value	-	0.04	0.000	0.02	-
The maximum density of the clot	389.0(289.0;444.5)	468.5(438.0;696.5)	493.0(462.0;573.0)	479.0(449.5;641.5)	0.001
P-value	-	0.005	0.000	0.001	-
The intensity of total clotting	7.9(6.1;11.8)	12.3(10.6;19.8)	11.1(9.8;13.0)	13.5(11.9;17.1)	0.001
P-value	-	0.005	0.02	0.001	-
The time of the beginning of fibrinolysis (min)	29.7(29.5;29.9)	-	-	-	

clotting time from 12.1(10.7;14.1)min to 6.1(4.2;7.3)min (*P*=0.000) and simultaneously increased the intensity of the coagulation drive from 19.3(10.8;21.9)U to 34.2(23.3;55.6) U (*P*=0.003). Clot polymerization occurred earlier than in the control stage [(16.1(14.2;17.3)min and 23.5(20.7;25.3)min, respectively, *P*=0.000)], but the intensity of clot polymerization did not differ significantly from the control stage. The use of this sorbent also reduced the time of formation of a fibrin-platelet clot (from 32.6(29.9;38.8min to 25.8(24.5;28.0)min, *P*=0.000), while increasing the maximum clot density (from 389.0(289.0;444.5)U to 493.0(462.0;573.0)U, *P*=.000). At the same time, the use of Aseptisorb-A allowed an increase in the intensity of total coagulation (from 7.9(6.1;11.8)U to 11.1(9.8;13.0)U, *P*=0.02). An increase in the density of the clot with Aseptisorb-A contributed to the formation of a stable clot, and the phenomena of fibrinolysis were not observed.

Aseptisorb-D effects

The influence of Aseptisorb-D on the dynamics of the processes of regulation of the blood aggregate state also had distinctive features. Thus, the time of the CPC was at the level of 1.0(1.0;1.0) min and did not differ from the control stage. The intensity of the CPC was 117.5(83.0;206.5)U versus 39.5(17.3;63.0)U in the control stage (*P*=0.001).The time to reach the thrombin constant was statistically reduced, compared to the control stage [(2.1(1.6;2.8)min and 5.9(4.6;7.0)min, respectively, *P*=0.000)], and the thrombin activity constant was at a higher level than the control stage [(71.8(54.5;125.0) U and 17.8(13.6;27.4)U, respectively, *P*=0.000). The use of Aseptisorb-D significantly reduced the blood clotting time (from 12.1(10.7;14.1)min to 4.2(2.5;5.4)min, *P*=0.000), while the intensity of the coagulation drive was 46.7(30.8;70.4)U. Clot polymerization occurred earlier than in the control stage [14.2(12.5;15.4)min and 23.5(20.7;25.3)min, respectively, *P*=0.000)], but the intensity of clot polymerization did not differ significantly from the control stage. The use of Aseptisorb-D reduced the time of formation of a fibrin-platelet clot (from 32.6(29.9;38.8)min to 28.7(26.0;29.5)min, *P*=0.02), while simultaneously making it possible to increase the maximum clot density (from 389.0(289.0;444.5)U to 479.0(449.5;641.5) U, *P*=0.001) and total coagulation intensity (from 7.9(6.1;11.8) to 13.5(11.9;17.1)U, *P*=0.001). When using Aseptisorb-D, the clot was also dense and the processes of fibrinolysis were not recorded.

Summing up the results of the experimental study, the most important finding for surgeons is that among all indicators of blood clotting processes there are 2 main ones: the time of blood clotting and the maximum density of the clot. Blood clotting time is a key indicator that reflects the transition of the liquid state of the blood to a gel-like state and coincides with the implementation of the thrombin explosion and is highly correlated with the time of reaching the peak concentration of thrombin in the thrombin generation test. The maximum density is the final qualitative characteristic of the entire process of thrombosis and reflects the resistance of the clot to external influences.[2-24] The use of the granulated sorbents Aseptisorb, Aseptisorb-A and Aseptisorb-D allows us to significantly accelerate the blood clotting time and increase the maximum clot density, which provides a theoretical justification for the effectiveness of these sorbents for surgical hemostasis.

Conclusion

Experimental studies have shown that the use of granular sorbents Aseptisorb, Aseptisorb-A, and Aseptisorb-D in varying degrees affects the links of platelet and coagulation hemostasis, providing acceleration of thrombosis processes while increasing the maximum density of the clot. These effects determine the effectiveness of the clinical use of these sorbents to stop various types of bleeding.

Experimental studies of the effect of granular sorbents on the system regulating the aggregate state of the blood using piezoelectric thromboelastography have shown that the use of Aseptisorb, Aseptisorb-A, Aseptisorb-D, and Sephadex G-50 can significantly reduce the time of blood clotting and increase the maximum clot density, which determines the possibility of the use of these sorbents in e*ndoscopic hemostatic* treatment for GDB.

Competing Interests

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

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Comparative Assessment of Inflammatory Reaction in Experimental Animals after Pleurodesis with Solutions of Hydrogen Peroxide and Talc

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Abstract

The aim of our research was to compare the nature and severity of the inflammatory process in the lungs, in the leaves of the visceral and parietal pleura, and in the adjacent subpleural tissues of the chest wall in experimental animals after pleurodesis with solutions of 3% and 6% hydrogen peroxide, and talc.

Methods and Results: The experiment was carried out on 200 Wistar rats, weighing 160-180 grams, 10 specimens in a subgroup, depending on the time of the experiment, i.e. 50 specimens in each study group, including the control group. The main criterion by which we determined the comparative characteristics of the effectiveness of talc and 3% and 6% solutions of hydrogen peroxide as preparations used for chemical pleurodesis in the rats was a morphological characteristic of inflammation. This criterion was confirmed by counting free cell populations in lung tissue (lymphocytes, macrophages, neutrophils, histiocytes). All comparison groups were characterized by a gradual increase in the number of lymphocytes, macrophages and histiocytes, ranging from minimum to maximum values, and by a gradual decrease in the number of neutrophils, starting with max and ending with minimum values. The number of lymphocytes, macrophages and histiocytes were increasing faster. But at the same time, for the most part, their number was lower after pleurodesis with 6% hydrogen peroxide. The minimum number of neutrophils and the fastest possible reduction in all cases was observed in pleurodesis with 6% hydrogen peroxide.

Conclusion: Pleurodesis with a 6% solution of hydrogen peroxide as a chemical agent significantly affects the quality of the inflammatory response, reducing its duration and severity in the organs and tissues of the rats' chests, compared with a solution of 3% hydrogen peroxide and talc. (**International Journal of Biomedicine. 2021;11(3):291-295.**)

Key Words: pneumothorax • pleurodesis • talc • hydrogen peroxide • free cell populations

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Introduction

According to the latest data, spontaneous pneumothorax (SP) has been identified in 6.2%–7.1% of patients with nonspecific lung diseases. This pathology tends to increase steadily, and currently there are about 15 patients per 100,000 inhabitants per year. It is important to note that the prevalence of SP is 7.4-18 cases per 100,000 men and 1.2-6 cases per 100,000 women per year. According to the results of several studies,[1] SP accounts for 11.2% of all acute pathology faced by thoracic surgeons. Note that this pathology is often found among people suffering from chronic obstructive pulmonary disease (COPD) - 26 cases per 100,000 population per year.[2] According to modern conceptions, the cause of nonspecific SP in 94.5% of cases is the destruction of emphysematous altered bulls, thereby once again confirming that SP is a complication of pulmonary emphysema and COPD.[1,3-5]

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The question of preventing the recurrence of SP remains relevant. A significant number of authors consider it necessary to use chemical pleurodesis in cases of pneumothorax occurrence, and even more in cases of its recurrence. Currently, various chemical agents are used for chemical pleurodesis: talc, olive oil, 40% glucose solution, hypertonic sodium chloride solution, acromycin, 96% alcohol solution and many others.[1,6-8]

An important and common drawback of the chemical agents used is the development of an excessive inflammatory response with severe pain and the development of various complications in the future.

Questions persist: What is the optimal chemical agent to perform pleurodesis? Taking the above data into consideration, we can claim that this scientific study is relevant.

The aim of our research was to compare the nature and severity of the inflammatory process in the lungs, in the leaves of the visceral and parietal pleura, and in the adjacent subpleural tissues of the chest wall in experimental animals after pleurodesis with solutions of 3% and 6% hydrogen peroxide, and talc.

Materials and Methods

The experiment was carried out on 200 Wistar rats, weighing 160-180 grams, 10 specimens in a subgroup, depending on the time of the experiment, i.e. 50 specimens in each study group, including the control group.

SP was simulated in rats under ether anesthesia by injection of air in the volume of 2 ml through a Velish needle.

After 1 hour under ether anesthesia, one of the chemical agents with a volume of 1.0 ml (hydrogen peroxide solution at concentrations of 6% or 3%, talc) was sprayed with a Velish needle and the air from the pleural cavity was removed. Then the animals were observed and killed in groups on Days 3, 5, 7, and 30 of the experiment. *In vivo* experiments were carried out in accordance with the legislation of the Russian Federation, in strict compliance with the European Convention for the protection of animals used for experimental and other purposes (Strasbourg, France, 1986), the provisions of Directive 210/63/EU of the European Parliament and the Council of the European Union of 22 September 2010 on the protection of animals used for scientific purposes (Article 27).

At opening of pleural cavities of the experimental animals, organs and tissues of the thorax were sampled for histological research. Pieces of lungs with adjacent parts of the chest wall were fixed in 10% neutral formalin and stained using standard histological techniques. Paraffin sections 6-7 microns thick after de-embedding (removing of paraffin) were stained with H&E for review.

In histological examination, we performed a comparative analysis of the severity of inflammatory changes in the interstitium of the lungs and commissure formation, depending on the agent used in pleurodesis.

The main criterion by which we determined the comparative characteristics of the effectiveness of talc and 3% and 6% solutions of hydrogen peroxide as preparations used for chemical pleurodesis in the rats was a morphological characteristic of inflammation. This criterion was confirmed by counting free cell populations in lung tissue (lymphocytes, macrophages, neutrophils, histiocytes).

Statistical analysis was performed using Microsoft Excel software package. For descriptive analysis, results are presented as median (Me), first quartile (25th percentile) and third quartile (75th percentile). A non-parametric Kruskal-Wallis test was used for comparisons of median values among groups.

Results

The results of descriptive statistics for free cell elements in tissues after chemical pleurodesis are presented in Table 1. The distribution of the compared values in most samples was abnormal.

The data in Table 2 indicate that the method of pleurodesis significantly affects the number of free cell elements. The differences between all pairs were estimated by the Kruskal-Walliss criterion as significant, as the significance levels were <0.05.

On Day 3 after pleurodesis with 6% hydrogen peroxide, the number of lymphocytes was less than after 3% hydrogen peroxide by 13.33% and after talc by 33.33%, but more than in the control group by 13.33%. The number of macrophages was greater than after 3% hydrogen peroxide by 8.33%, less than after talc by 25%, and more than in the control group by 25%. The number of neutrophils was less than after 3% hydrogen peroxide by 21.15% and after talc by 25%, but more than in the control group by 48%. The number of histiocytes on Day 3 after pleurodesis with 6% hydrogen peroxide was less than after 3% hydrogen peroxide by 8.33% and after talc by 16.66%, but more than in the control group by 8.33%.

On Day 5 after pleurodesis with 6% hydrogen peroxide, the number of lymphocytes was less than after 3% hydrogen peroxide by 8.33% and after talc by 20.83%, but more than in the control group by 45.83%. The number of macrophages was greater than after 3% hydrogen peroxide by 20.0%, less than after talc by 6.66%, and more than in the control group by 46.66%. The number of neutrophils was less than after 3% hydrogen peroxide by 27.5% and after talc by 37.5%, but more than in the control group by 25%. The number of histiocytes on Day 5 after pleurodesis with 6% hydrogen peroxide was less than after 3% hydrogen peroxide by 18.18% and after talc by 27.77%, but more than in the control group by 36.36%.

On Day 7 after pleurodesis with 6% hydrogen peroxide, the number of lymphocytes was more than after 3% hydrogen peroxide by 11.53% and after talc by 3.85% and more than in the control group by 73.5%. The number of macrophages was greater than after 3% hydrogen peroxide by 6.25%, less than after talc by 12.5%, and more than in the control group by 43.75%. The number of neutrophils was less than after 3% hydrogen peroxide by 33.3% and after talc by 42.2%, but more than in the control group by 15.5%. The number of histiocytes on Day 7 after pleurodesis with 6% hydrogen peroxide was less than after 3% hydrogen peroxide by 18.18% and after talc by 36.36%, but more than in the control group by 63.63%.

On Day 10 after pleurodesis with 6% hydrogen peroxide, the number of lymphocytes was more than after 3% hydrogen peroxide by 8.95%, less than after talc by 8.95% and more than in the control group by 68..65%. The number of macrophages was greater than after 3% hydrogen peroxide by 10.52%, less than after talc by 15.78%, and more than in the control group by 52.63%. The number of neutrophils was less than after 3% hydrogen peroxide by 19.23% and after talc by 30.76%, but more than in the control group by 11.53%. The number of histiocytes on Day 10 after pleurodesis with 6% hydrogen peroxide was less than after 3% hydrogen peroxide by 13.33% and after talc by 26.66%, but more than in the control group by 46.66%.

On Day 30 after pleurodesis with 6% hydrogen peroxide, the number of lymphocytes was less than after 3% hydrogen peroxide by 6%, after talc by 28%, and by 58% more than in the control group. The number of macrophages was greater than after 3% hydrogen peroxide by 9.09%, less than after talc by 13.63%, and more than in the control group by 59.09%. The number of neutrophils was less than after 3% hydrogen peroxide by 16.66% and after talc by 29.16%, but more than in the control group by 8.33%. The number of histiocytes on Day 10 after pleurodesis with 6% hydrogen peroxide was less than after 3% hydrogen peroxide, on average, by 11.11% and after talc by 22.22%, but by 61.11% more than in the control group.

Table 1.

Descriptive statistics for free cell elements in tissues after chemical pleurodesis

Days	Parameter	Talc			3% hydrogen peroxide			6% hydrogen peroxide			Control group		
		Me	25	75	Me	25	75	Me	25	75	Me	25	75
3	lymphocytes	15	14	15	12	12	12	10	9	10	8	7	8
	macrophages	12	11	13	8	8	9	9	9	10	6	6	7
	neutrophils	52	52	53	50	49	50	39	39	40	14	14	14
	histiocytes	10	9	10	8	7	8	6	7	8	4	3	4
5	lymphocytes	24	23	24	21	20	21	19	18	19	8	8	9
	macrophages	15	14	15	11	10	11	14	13	14	6,5	6	7
	neutrophils	40	40	40	37.5	37	38	25	25	26	15	15	15
	histiocytes	11	11	13	10	9	10	8	7	8	4	4	4
7	lymphocytes	25	25	26	23	22	23	26	26	27	7	7	7
	macrophages	16	16	17	13	13	13	14	14	14	7	7	7
	neutrophils	33	33	34	30	30	30	19	18	19	14	13	14
	histiocytes	14	14	14	12	11	12	10	10	10	3	3	3
10	lymphocytes	33.5	33	34	28	27	28	31	31	31	8	7	8
	macrophages	19	19	19	13.5	13	14	16	16	16	6	6	6
	neutrophils	26	33	34	22.5	22	23	18	18	18	15	15	15
	histiocytes	15	15	16	13	13	14	11	11	12	4	4	5
30	lymphocytes	50	50	51	39	38	39	36	36	37	7	7	8
	macrophages	22	21	22	17	16	17	19	18	19	6	6	7
	neutrophils	24	24	24	21	21	21	17	16	17	15	15	15
	histiocytes	18	17	18	16	16	16	14	13	14	3	2	3

Table 2.

The results of data processing by Kruskal-Walliss criterion (P-level)

Comparison groups	Lymphocytes					Macrophages					Neutrophils					Histiocytes				
	Day 3	Day 5	Day 7	Day 10	Day 30	Day 3	Day 5	Day 7	Day 10	Day 30	Day 3	Day 5	Day 7	Day 10	Day 30	Day 3	Day 5	Day 7	Day 10	Day 30
All	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Talc – 3% hydrogen peroxide	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Talc– 6% hydrogen peroxide	0.001	0.001	0.009	0.002	0.001	0.001	0.005	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Talc – control	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
3% hydrogen peroxide - 6% hydrogen peroxide	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
3% hydrogen peroxide –control	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
6% hydrogen peroxide –control	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001

All comparison groups were characterized by a gradual increase in the number of lymphocytes, macrophages and histiocytes, ranging from minimum to maximum values, and by a gradual decrease in the number of neutrophils, starting with max and ending with minimum values. The predominance of neutrophilic leukocytes over other cell populations indicates an acute inflammatory reaction to the introduction of the drug; a further increase in the level of lymphocytes, macrophages, histiocytes and, accordingly, a decrease in the level of neutrophils indicates the transition of acute inflammation to chronic. By comparing the dynamics of the number of analyzed free cell elements, we found that the number of lymphocytes, macrophages and histiocytes were increasing faster. But at the same time, for the most part, their number was lower after pleurodesis with 6% hydrogen peroxide. The minimum number of neutrophils and the fastest possible reduction in all cases was observed in pleurodesis with 6% hydrogen peroxide. In the comparison group, only fluctuations in the number of the initial level of free cell elements were observed.

Conclusion

- The dynamics of the number of free cell elements in all comparison groups was estimated as stereotypical.

- The differences in all the investigated pairs were evaluated by Kruskal-Walliss criterion as significant.

- Kruskal-Walliss analysis suggests that the method of pleurodesis significantly affects the number of free cell elements involved in the inflammatory response.

- Pleurodesis with a 6% solution of hydrogen peroxide as a chemical agent significantly affects the quality of the inflammatory response, reducing its duration and severity in the organs and tissues of the rats' chests, compared with a solution of 3% hydrogen peroxide and talc.

Competing Interests

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

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Direct Pharmacological Correction of Oxidative Stress in Rat Kidneys Does Not Facilitate Diabetic Nephropathy

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Abstract

The aim of this study was to evaluate the effect of alpha-tocopherol acetate (ATA) on the activity of free-radical oxidation (FRO) in renal tissue and renal function in rats with experimental streptozotocin (STZ)-induced diabetes mellitus (DM).

***Methods and Results*:** Experiments were conducted on 22 male Wistar rats aged 60-100 days and weighing 250-300 g. The animals were divided into two groups: Group 1 (control) and Group 2 (experimental). To induce DM, the animals were injected intraperitoneally 1ml of STZ solution in the citrate buffer at a dose of 65 mg/kg. For more selective modeling of type 2 DM, the rats were previously injected with an intraperitoneal solution of cytoflavin based on a nicotinamide dose of 115 mg/kg. In Croup 2, ATA was administered in the period from the fifth to eighth weeks, inclusive, intragastrically through a tube at a daily dose of 300 mg/kg.

Experiments showed that after a 4-week course of ATA, the concentration of thiobarbiturate-reactive products in the kidney tissues of the rats in Group 2 was 5.3 times lower than in Group 1. The activity of all antioxidant enzymes did not differ between the two groups. In both groups, during all 8 weeks of the experiment, the levels of renal excretion of glucose, protein, and creatinine significantly exceeded the initial level, while the level of diuresis remained stable.

Conclusion: The long-term administration of ATA in experimental streptozotocin (STZ)-induced DM is accompanied by a significant suppression of the activity of the FRO processes in the kidneys, but does not lead to an improvement in the course of diabetic nephropathy. (**International Journal of Biomedicine. 2021;11(3):296-300.**)

Key Words: kidneys • diabetic nephropathy • tocopherol acetate • oxidative stress

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Abbreviations

ATA, alpha-tocopherol acetate; **CAT**, catalase; **DM**, diabetes mellitus; **DN**, diabetic nephropathy; **FRO,** free-radical oxidation; **GPx**, glutathione peroxidise; **OS**, oxidative stress; **ROS**, reactive oxygen species; **SOD**, superoxide dismutase, **TBRP**, thiobarbiturate-reactive products.

Introduction

Numerous studies have proposed that OS plays a crucial role in the progression and severity of diabetic nephropathy (DN).[1-3] The activation of FRO in the kidneys, against the background of diabetes mellitus (DM), has been shown in many works, including our previous study.[4] However, the mechanisms that develop oxidative damage to the renal glomerulus are quite diverse. They can be direct and indirect. It is generally accepted that in DM, glucose and its metabolites in urine can directly suppress the activity of cellular antioxidants, such as glutathione.[5-8] Numerous studies have shown that in DM, there exists an accumulation of advanced glycated end-products,[5,9,10] increased OS,[11] and enhanced angiotensin II levels.[5,12] The adverse effects of most of those factors have often been linked to the generation of ROS.[3,13] Many sources

of ROS contribute to increased OS; however, NADPH oxidases (Nox) and their catalytic subunit are the only known enzyme family solely dedicated to producing ROS.[3,5,13-15] Furthermore, Nox isoforms are upregulated in the presence of high glucose. The resulting ROS contribute to damage to podocytes, causing nephropathy.[1,16-18] Considering the above, it is obvious that pharmacological correction of OS in the kidneys in DM may have a beneficial effect on the course of DN. However, the question remains unresolved: Suppressing which of the above mechanisms of intrarenal OS can be most effective in treating DN? To understand this issue, in the first stage of our study we decided to evaluate the effectiveness of correcting the direct prooxidant effect of hyperglucosuria, as one of the first mechanisms in the formation of OS in DN. For this, we chose the classical direct antioxidant ATA as a pharmacological tool.

The aim of this study was to evaluate the effect of ATA on the activity of FRO in renal tissue and renal function in rats with experimental streptozotocin (STZ)-induced DM.

Materials and Methods

Experiments were conducted on 22 male Wistar rats aged 60-100 days and weighing 250-300g. The work with animals was carried out in accordance with the principles of humanism laid down in the directives of the European Community (86/609/EEC) and the Declaration of Helsinki, in accordance with the "Animal experimentation legislations."

The animals were divided into two groups. In Group 1 (control) (n=10), to induce DM, the animals were injected intraperitoneally 1ml of STZ solution in the citrate buffer at a dose of 65mg/kg. For more selective modeling of type 2 DM, the rats were previously injected with an intraperitoneal solution of cytoflavin based on a nicotinamide dose of 115 mg/kg..[19] In Group 2 (experimental) (n=12), DM was simulated in a similar way and ATA was administered. In preliminary studies, we have shown that typical signs of nephropathy in rats, including OS in the kidneys, develop as early as 4 weeks after the administration of STZ.[20] At the same time, we also found that in rats with 8-month DM, pathological changes in the kidneys become extremely pronounced and, most likely, irreversible.[21] The results obtained show that the most reliable assessment of the effectiveness of pharmacological correction of DN is possible mainly in the early stages of DN, and therefore the total duration of the experiment in this study was 8 weeks (4 weeks of *STZ*-induced DM, then another 4 weeks - treatment). In Croup 2, ATA was administered in the period from the fifth to eighth weeks, inclusive, intragastrically through a tube at a daily dose of 300 mg/kg. This dose was chosen based on the results of our previous experiments to study the effect of ATA on the course of experimental oxalate nephrolithiasis.[22] In both groups, before starting diabetes modeling, and then weekly, we determined the concentration of glucose, protein, and creatinine in the urine, and their urinary excretion was calculated.

In urine, the concentration of glucose, protein, and creatinine was determined on the automatic biochemical analyzer DIRUICS-T240 using commercial biochemical kits (DIAKON-DS, Russia). After 8 weeks of the experiment, the rats were euthanized under ethereal anesthesia and both kidneys were extracted, one of which was used for morphological research, and the other one was used to determine the biochemical markers of oxidative stress.

The activity of FRO in the kidneys of the rats was assessed by measuring the concentration of TBRP in the renal tissue homogenate and the activity of antioxidant enzymes (SOD, CAT, GPx).[15,22] The morphological study was carried out according to the scheme we approved in previous experiments.[20]

Statistical analysis was performed using using a special program Statistica 13.3.1 (license JPZ906I448517FAACD-K). The normality of distribution of continuous variables was tested by one-sample Kolmogorov-Smirnov test. Continuous variables with normal distribution were presented as mean (standard error of the mean [SEM]); non-normal variables were reported as median (Me) and interquartile range (IQR; 25th to 75th percentiles). The Mann-Whitney U Test was used to compare the differences between the two independent groups. 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

Experiments showed that after a 4-week course of ATA, the TBRP concentration in the kidney tissues of the rats in Group 2 was 5.3 times lower than in Group 1. The activity of all antioxidant enzymes did not differ between the two groups (Table 1).

Table 1.
Indicators of activity of FRO in the kidneys of the rats

Variable	Group 1 (Control)	Group 2 (Experiment)	*P*-value
TBRP concentration (μmol/mg)	10.1 (8.3;13.5)	1.9 (1.6;2.3)	<0.0000
CAT activity (%)	9.8 (6.2;27.1)	9.5 (7.7;24.1)	>0.05
SOD activity (%)	6.8 (4.7;8.5)	6.5 (4.8;10.3)	>0.05
GPx activity (%)	34.7 (31.4;37.3)	37.2 (34.0;40.0)	>0.05

In Group 1, during all 8 weeks of the experiment, the levels of renal excretion of glucose, protein, and creatinine significantly exceeded the initial level, while the level of diuresis remained stable (Table 2). In Group 2, despite the course of treatment, the described parameters did not differ from Group 1.

The results of a morphological study showed that after 8 months of *STZ*-induced DM, the kidney glomeruli of the Group 1 animals were enlarged. In addition, there was a significant expansion of the intercapillary space due to the accumulation of Schiff-positive mesangium and connective tissue.

Table 2.

Indicators of excretory kidney function in the two groups

	Diuresis (ml/day)		Excretion of glucose (μmol / day)		Protein excretion (mg / day)		Excretion of creatinine (μmol / day)	
	Group 1 (Control)	Group 2 (Experiment)	Group 1 (Control)	Group 2 (Experiment)	Group 1 (Control)	Group 2 (Experiment)	Group 1 (Control)	Group 2 (Experiment)
Initial level	1.6 (1.0;2.0)	1.2 (1.0;1.8)	0.1 (0.02;0.38)	0.1 (0.02;0.53)	7.3 (1.7;7.9)	6.0 (4.2;9.5)	18.3 (1.9;26.1)	18.1 (12.822.7)
Week 1	1.8 (1.4;2.2)	2.0 (1.5;2.8)	10.8 (28.6;12.2) *P*=0.003	10.0 (5.3;15.5) *P*=0.002	10.9 (8.7;24.4) *P*=0.016	13.5(9.9;15.3) *P*=0.004	32.9 (28.5;41.0) *P*=0.016	37.1 (26.2;48.6) *P*=0.008
Week 2	1.6 (1.2;2.4)	2.0 (1.1;2.4)	0.8 (0.4;3.6) *P*=0.026	1.7(0.8;3.6) *P*=0.003	7.7 (6.1;14.1)	10.5 (7.6;12.4) *P*=0.012	25.2 (23.6;34.8) *P*=0.033	35.1 (21.4;39.2) *P*=0.006
Week 3	1.6 (1.4;2.0)	1.7 (1.1;1.9)	1.3 (0.8;1.9) *P*=0.009	1.5 (1.2;2.2) *P*=0.013	10.8 (7.3;13.5) *P*=0.013	9.3 (7.8;11.5) *P*=0.034	46.6 (24.1;51.3) *P*=0.013	42.7 (31.6;44.7) *P*=0.003
Week 4	1.9 (1.6;2.2)	1.9 (1.3;2.1)	1.1 (1.0;2.3) *P*=0.028	0.8 (0.5;1.6) *P*=0.019	10.2 (8.1;15.9) *P*=0.022	10.0 (8.5;11.2) *P*=0.015	36.3 (29.1;52.3) *P*=0.017	35.2 (26.2;45.6) *P*=0.002
Week 5	1.8 (1.2;2.8)	1.7 (1.0;1.9)	1.7 (1.0;1.9)	0.6 (0.3;1.6) *P*=0.015	10.9 (6.9;12.1) *P*=0.017	9.6 (6.1;12.2)	30.4 (11.5;46.8) *P*=0.047	21.1 (10.6;32.6)
Week 6	1.6 (0.8;2.2)	1.4 (0.8;2.0)	2.2 (1.6;4.8) *P*=0.009	2.2 (1.5;3.2 *P*=0.002	8.4 (4.6;9.7)	7.9 (6.3;11.9)	29.5 (18.4;50.5) *P*=0.022	30.4 (18.9;39.6) *P*=0.023
Week 7	1.2 (1.0;1.4)	1.0 (0.9;1.5)	1.2 (0.8;1.3) *P*=0.037	1.4 (1.0;2.2) *P*=0.012	7.4 (7.1;10.7) *P*=0.047	7.1 (5.6;9.6)	33.1 (24.8;49.9) *P*=0.009	34.2 (28.1;48.0) *P*=0.002
Week 8	1.3 (0.8;2.0)	1.0 (0.7;1.5)	1.5 (0.8;2.2) *P*=0.017	1.7 (0.9;2.7) *P*=0.010	10.3 (7.4;13.5) *P*=0.017	11.6 (5.9;14.5)	34.2 (21.0;49.7) *P*=0.013	35.9 (19.1;45.7) *P*=0.019

P – level of significance with the initial data

The basal membranes of the glomeruli capillaries were significantly thickened, the capillary lumen was narrowed. The capsule of the kidney glomeruli looked thickened. Glomeruli capillaries were full-blooded. In the kidney interstitium, there were foci of lymphoplasmacytic infiltration. The tubular lumens were expanded; the basal membranes of the tubulars were thickened. Nephrocytes were in a state of hyaline-drop dystrophy. The walls of the arteries were thickened; the elastic membranes were hyperplastic. The blood vessels were in a state of plethora (Fig 1).

In the Group 2 animals, the size of the kidney glomeruli decreased slightly, compared to Group 1; the intercapillary space was expanded due to the accumulation of Schiff-positive mesangium and connective tissue. The basal membranes of the glomeruli capillaries were thickened, the capillary lumen was narrowed. The capsule of the kidney glomeruli looked thickened. Glomeruli capillaries were full-blooded. In the kidney interstitium, there were single foci of lymphoplasmacytic infiltration. The tubular lumens were expanded; the basal membranes of the tubulars were thickened. Nephrocytes were in a state of hyaline-drop dystrophy. The walls of the vessels were moderately thickened; the elastic membranes were hyperplastic (Fig.2).

Table 3 presents the quantitative indicators of the morphometric study of the kidneys of both groups of rats. Statistically significant differences between groups were not identified.

The question of whether oxidative stress is primarily responsible for the diabetic complications was extensively investigated, but remained unanswered. The results obtained, to a certain extent, were unexpected. On the one hand, ATA exerted its direct antioxidant effect in the kidneys, as evidenced by a fivefold decrease (relative to the control group) in the concentration of TBRP, the main marker of membrane phospholipid peroxidation. At the same time, ATA had no effect on the activity of antioxidant enzymes, which is also in good agreement with the direct non-enzymatic nature of the antioxidant effect of ATA.

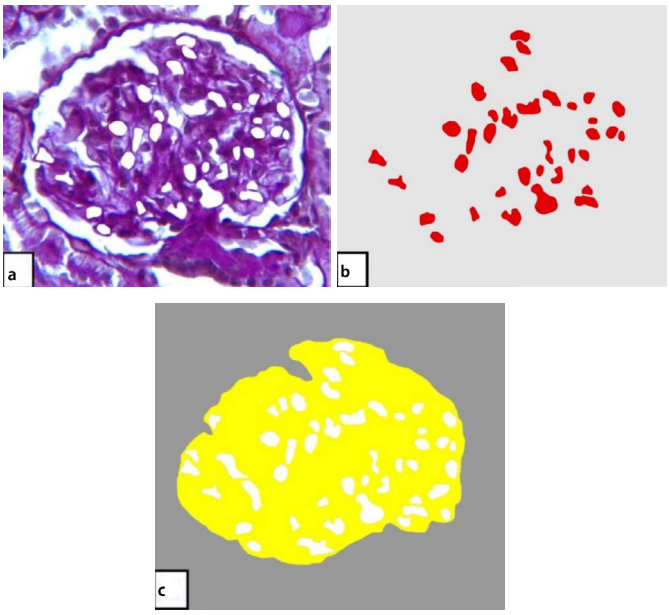

Fig. 1. *Control group.*
(a) - Kidney glomerulus; (b) - Luminal narrowing of capillaries; (c) - Increased mesangial area. McManus staining method; computer processing of photomicrographs (×1200).

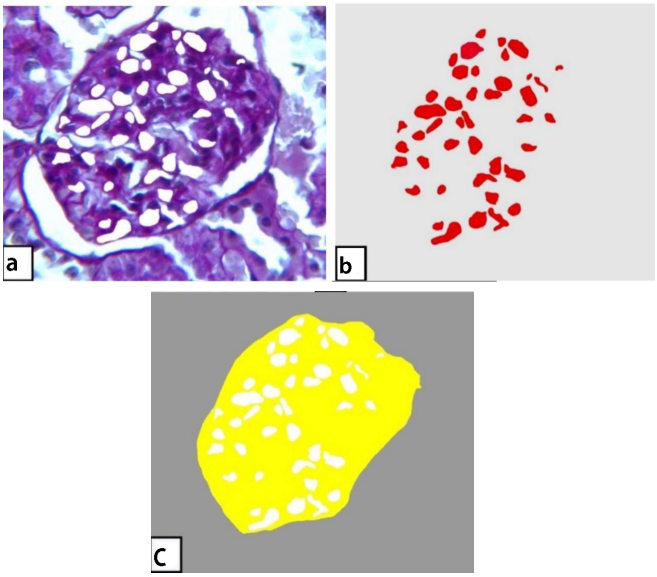

Fig. 2. *Experimental group.*

(a) - Kidney glomerulus; (b) - Luminal narrowing of capillaries; (c) - Increased mesangial area. McManus staining method; computer processing of photomicrographs (×1200).

Table 3.

The quantitative indicators of the morphometric study of the kidneys of both groups of rats

Indicators	Group 1 (Control)	Group 2 (Experiment)	P-level
The area of the renal glomeruli (μm²)	7089.5±262.7	6316.3±115.5	>0.05
The total area of blood vessels in the glomerulus (μm²)	1064.6±115.5	953.3±112.5	>0.05
Glomerular capillary lumen area (μm²)	22.65±1.8	23.25±2.1	>0.05
Mesangium area in the glomeruli (μm²)	5396.6±85.8	4548.1±115.5	>0.05
The number of podocytes in the glomerulus	28.2±2.9	34.3±2.3	>0.05

At the same time, ATA did not cause any significant changes in the development of experimental pathology. A high level of proteinuria and glomerular filtration rate persisted, and morphological changes in tissues and cells of the renal glomeruli were identical to the control.

The results obtained suggest that the suppression of the direct component of the oxidative effect of glucose and its metabolic products is insufficient to normalize the structure and function of the renal filtration barrier. It is possible that the mediated mechanisms of oxidation make a much more significant contribution to the development of nephropathy. In this regard, the search for effective methods of pharmacological correction of DN implies further study of the effects of OS on the development of nephropathy.

It should be noted that the effect of ATA on the course of DN has been studied by various researchers. There are a number of studies showing the effectiveness of this drug as an antioxidant in the treatment of DN.[23-25] At the same time, other authors believe that the role of ATA in the correction of DN is controversial and needs to be studied further. The results of our study showed that ATA, while exerting a pronounced suppression of FRO in the kidneys in experimental DM, did not contribute to the correction of DN.

In conclusion, the long-term administration of ATA in experimental streptozotocin (STZ)-induced DM is accompanied by a significant suppression of the activity of the FRO processes in the kidneys, but does not lead to an improvement in the course of DN.

Competing Interests

The authors declare that they have no competing interests.

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SHORT
COMMUNICATION

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The Diagnostic Value of the Leukocyte Shift Index in Purulent-Septic Rhinosinusogenic Complications in Children

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Abstract

The aim of this study was to investigate the diagnostic value of the leukocyte shift index (LSI) in inflammatory pathology of the paranasal sinuses (PNS) with the rhinosinusogenic orbital complications (RSOC) in pediatric patients.

Methods and Results: The study involved 50 patients (26 boys and 24 girls) with diseases of the PNS and RSOC (reactive edema of the eyelids, orbital tissue, and purulent-septic complications of the eyelids and orbit) aged from 1 to 17 years (mean age of 6.66±0.63 years). Group 1 included 29 patients (16 boys and 13 girls) with reactive edema of the eyelids and orbital tissue. Group 2 included 21 patients (10 boys and 11 girls) with purulent-septic RSOC. As a marker for determining the activity of the inflammatory process and the disorders of the immunological reactivity of the body, LSI (leukocyte shift index) was calculated. In general, the LSI value was 1.61±0.21 in Group 1 and 3.45±0.49 in Group 2 (P=0.001). Among patients aged between 3 and 12 years, the LSI was 1.66±0.30 in Group 1 and 3.93±0.79 in Group 2 (P=0.012). The results obtained indicate that LSI can be used to predict purulent-septic RSOC in inflammatory diseases of PNS in patients aged between 3 and 12 years. LSI values from 1.36 to 1.96 may predict the development of reactive edema of the eyelids and orbital tissue; from 3.14 to 4.72 - the development of purulent-septic complications of the eyelids and orbit.

Conclusion: The results obtained can be useful in predicting the clinical course of the RSOC in inflammatory pathology of PNS in patients in the age group of 3-12 years. (**International Journal of Biomedicine. 2021;11(3):301-304.**)

Key Words: paranasal sinuses • rhinosinusogenic orbital complications • leukocyte shift index

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Abbreviations

CT, computed tomography; **LSI**, leukocyte shift index; **MRI**, magnetic resonance imaging; **PNS**, paranasal sinuses: **RSOC**, rhinosinusogenic orbital complications.

Introduction

Currently, sinusitis is one of the most common ENT diseases in pediatric practice. Timely diagnosis of sinusitis and its complications is very important.[1] Inflammatory diseases of the orbit in 40%-80% of cases are of rhinosinusogenic origin in adults and in 43% of cases in children. The prevalence of rhinosinusogenic orbital complications (RSOC), according to the data of the department of pediatric otolaryngology, is 12%, requiring a multidisciplinary approach to the tactics of treating pediatric patients.[2] Orbital complications rank first among all serious complications of acute sinusitis.[3,4] The anatomical features of the paranasal sinuses (PNS), their direct connection with the orbit, contribute to the rapid development of formidable septic complications.[5] In children, especially young children, diagnosis is often difficult, according to objective data and the results of additional research methods.

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The aim of this study was to investigate the diagnostic value of the leukocyte shift index (LSI) in inflammatory pathology of the PNS with the RSOC in pediatric patients.

Materials and Methods

The study involved 50 patients (26 boys and 24 girls) with diseases of the PNS and RSOC (reactive edema of the eyelids, orbital tissue, and purulent-septic complications of the eyelids and orbit) aged from 1 to 17 years (mean age of 6.66±0.63 years). All patients were treated in the department of pediatric otolaryngology at the Regional Clinical Hospital No.2 of Tyumen in the period from 2018 to 2019. Group 1 included 29 patients (16 boys and 13 girls) with reactive edema of the eyelids and orbital tissue. Group 2 included 21 patients (10 boys and 11 girls) with purulent-septic RSOC. As a marker for determining the activity of the inflammatory process and the disorders of the immunological reactivity of the body, we chose LSI,[6] calculated taking into account the parameters of the general blood test.[7]

The LSI value of 1.96±0.56 is considered normal. The LSI values are influenced by the characteristics of the physiological state of the organism, depending on age (the first cross at 5 days and the second at 5 years).

All children were admitted on an emergency basis with complaints of difficulty in nasal breathing, redness, swelling of the eyelid skin, narrowing of the palpebral fissure, headaches, pain in the projection of the sinuses, unilateral disturbance of the outflow of discharge from the nose, hyperthermia, anxiety, and sleep disturbance. All children were examined by an otorhinolaryngologist, pediatrician, and ophthalmologist, as well as by a neurologist, anesthesiologist, and neurosurgeon, according to the indications. Upon admission, patients underwent clinical and laboratory diagnostics, radiography, and CT, as well as MRI of the PNS, orbit, and brain, according to indications. CT or MRI with contrast was performed for diagnostic purposes to exclude intraorbital and intracranial complications. Surgical treatment of the PNS diseases (catheterization, endoscopic sinusitis, orbitotomy) was performed according to indications. A course of intensive antibiotic therapy, heparin therapy, and local therapy was carried out.

Statistical analysis was performed using the statistical software STATISTICA (v10.0, StatSoft, USA). The normality of distribution of continuous variables was tested by one-sample Kolmogorov-Smirnov test. Continuous variables were presented as mean ± SEM. Means of 2 continuous normally distributed variables were compared by independent samples Student's t test. A value of $P<0.05$ was considered significant.

The study was conducted in accordance with ethical principles of the WMA Declaration of Helsinki (1964, ed. 2013) and approved by the Tyumen State Medical University Ethics Committee. Written informed consent was obtained from the patient/parent/guardian/ relative of each patient.

Results and Discussion

In the present study, in general, the LSI value was 1.61±0.21 in Group 1 and 3.45±0.49 in Group 2 (P=0.001). However, a comparative analysis of LSI in the two groups, according to the age subgroups, did not show significant differences (Table 1, due to the small number of observations, but revealed significant fluctuations in this indicator: from 0.75 to 1.83 in Group 1 and from 1.88 to 4.65 in Group 2. In addition, in patients in the age subgroup of 1-2 years, this indicator was not informative in terms of predicting the development of the RSOC: rhinogenic reactive edema of the eyelids and orbital tissue developed with LSI of 1.54±0.23, and septic complications of the eyelids and orbit - with LSI of 1.88±0.53 (P>0.05).

Table 1.

LSI values in Croups 1 and 2 according to the age subgroups

Age subgroups (yrs)	Mean age, yrs		LSI		*P*-value
	Group 1	Group 2	Group 1	Group 2	
1-2 (n=9)	1.71±0.20 (n=7)	1.04±0.17 (n=2)	1.54±0.23	1,88±0.53	0.714
3-4 (n=15)	3.55±0.17 (n=12)	3.67±0.23 (n=3)	1.67±0.48	4.65±1.32	0.055
5-7 (n=7)	5.80±0.42 (n=3)	5.75±0.55 (n=4)	0.75±0.01	2.86±1.35	0.193
8-12 (n=8)	9.80±0.82 (n=5)	9.5±0.99 (n=3)	1.83±0.44	4.63±2.19	0.265
13-17 (n=11)	14.0±1.41 (n=2)	14.29±0.45 (n=9)	1.77±0.12	3.26±0.22	0.061

Particular attention should be paid to LSI indicators in the age group of 3-12 years. The data obtained indicate that children at this age are more likely to suffer from inflammatory diseases of the PNS and have RSOC. Thus, among patients aged between 3 and 12 years, the LSI was 1.66±0.30 in Group 1 and 3.93±0.79 in Group 2 (P=0.012). The results obtained indicate that LSI can be used to predict purulent-septic RSOC in inflammatory diseases of PNS in patients aged between 3 and 12 years. LSI values from 1.36 to 1.96 may predict the development of reactive edema of the eyelids and orbital tissue; from 3.14 to 4.72 - the development of purulent-septic complications of the eyelids and orbit. LSI values can complement a number of criteria for determining the indications for early surgical intervention and, the extent of it, as well as for the prevention of intraorbital and intracranial complications. To demonstrate the possibility of using LSI, we present two clinical cases.

Case Presentation 1

A 10-year-old girl was admitted on 11.26.2018 to the department of pediatric otolaryngology for emergency indications. Complaints: nasal congestion, edema of the lower and upper eyelids of the right eye.

Anamnesis morbi

According to the mother's words, the child fell ill on

11.23.2018, when swelling of the eyelids of the right eye appeared against the background of a purulent runny nose. Vasoconstrictor nasal drops were used in the treatment. According to her mother, following the treatment, the runny nose stopped.

Anamnesis vitae

Child from second pregnancy, first childbirth, delivery at term. Body weight at birth -3600g. Scheduled vaccinations. The patient grew and developed according to her age. Past diseases: acute respiratory infections, chickenpox. History of allergies is not burdened.

Clinical Findings, Diagnostic Assessment, and Treatment

General condition is moderate. Consciousness is clear. Body temperature - 36.6°C. The mucous membranes are clean and moist. The skin cover is clean, physiological color. Tongue is pink and moist. Pharynx: mucous membranes are pink and clear; the soft palate is mobile, with no plaque. The lymph nodes are intact. No peripheral edema. The respiratory rate – 26 breaths per minute. The heart rate is 84bpm. The abdomen is soft; the liver and spleen are not palpable. Urination is not disturbed. The stool is normal.

Right eye: The palpebral fissure is sharply narrowed. Edema, hyperemia of the upper and lower eyelids. The eyeball is fully mobile.

Nose: With anterior rhinoscopy on the right, the nasal mucosa is hyperemic, sharply edematous; in the nasal passages, there is scanty free discharge on the right, pronounced edema of the mucous membrane of the inferior turbinate; breathing is difficult. With anterior rhinoscopy on the left, the mucous membranes of the nasal conchas are pink, common nasal passage is visible, there is mucous discharge. The nasal septum lays along the midline.

Ears: AS and AD: The eardrum is gray, the cone of light is preserved, and the identification markings are visible.

LSI-1.8.

Ophthalmologist's examination: "Acute maxillary sinusitis. Reactive edema of the eyelids of the right eye."

This clinical case indicates that the value of ISL of 1.8U in purulent-inflammatory disease of the PNS is associated with the development of reactive edema of the eyelids.

Case Presentation 2

A 5-year-old boy was admitted to the department of pediatric otolaryngology on April 30, 2018, for emergency indications. Complaints: difficulty in nasal breathing, headache, fever (39.0°C), redness and edema of the right eyelids, narrowing of the palpebral fissure on the right, protrusion of the right eye anteriorly out of the orbit.

Anamnesis morbi

According to the mother, the child fell ill on 24.04.18, when a runny nose and difficulty in nasal breathing appeared. On the day of admission, there was a headache, swelling of the eyelids of the right eye, the eye is closed, temperature of 39°C.

Anamnesis vitae

Child from full-term pregnancy, body weight at birth – 3800g. The patient grew and developed according to his age. Scheduled vaccinations. Past diseases: acute respiratory infections. History of allergies is not burdened.

Clinical Findings, Diagnostic Assessment, and Treatment

Body weight is 23kg. The general condition is severe, due to the underlying disease, and symptoms of intoxication. Consciousness is clear. Body temperature - 39.0°C. The mucous membranes and skin cover are clean. Tongue is pink and moist. Pharynx: mucous membranes are pink and clear; the soft palate is mobile, with no plaque. Submandibular lymph nodes are enlarged up to 2 cm, moderately painful. The respiratory rate – 26 breaths per minute. The heart rate is 100 bpm. Breathing through the nose is difficult. The vesicular breathing is heard over the thorax. Heart sounds are sonorous, rhythmic. The abdomen is soft; the liver and spleen are not palpable. Urination is not disturbed. The stool is normal.

Right eye: Edema, infiltration of the eyelid skin. The eye slit is sharply narrowed, the eyeball is marked by restriction of mobility, exophthalmos.

Nose: The nasal breathing is difficult: pronounced swelling of the mucous nasal concha on the right, no discharge. On the right, the mucous membrane of the turbinates is sharply hyperemic, the nasal passage is narrowed; there is no free discharge. The nasal septum is in the middle position.

Oropharynx: Mucous membranes are pink and clear, with no plaque. Palatine tonsils of normal size.

Ears: The shape and size of the auricles, parotid and mastoid areas on both sides are not changed. External auditory canals are not changed; there is no discharge. The eardrum of AS and AD is light gray, the cone of light is preserved, and the identification markings are visible.

LSI=4.0. The patient was examined by ophthalmologist, maxillofacial surgeon, pediatrician, anesthesiologist, and neurologist. Clinical diagnosis: "Acute right-sided purulent polysinusitis. Rhinogenic phlegmon of the right orbit." Surgical treatment: endoscopic polysinusotomy, orbitotom. A course of antibiotic therapy, heparin therapy, and local treatment was carried out.

This clinical case indicates that the value of ISL of 4.0 in purulent-inflammatory disease of the PNS was associated with the development of purulent-septic complication - orbital phlegmon.

The presented patients were discharged with recovery under the supervision of an ENT doctor on an outpatient basis.

Conclusion

Domestic scientists have shown the role of leukocyte indices in assessing the body's immunological reactivity, the severity of endogenous intoxication, and its complications.[7,8] This study demonstrates the diagnostic significance of LSI in predicting the clinical course of the RSOC in inflammatory pathology of PNS in patients in the age group of 3-12 years. The development of reactive edema of the eyelids and orbital tissue is predicted when the LSI is from 1.36 to 1.96; purulent-septic lesions of the eyelids and orbital tissue- from 3.14 to 4.72.

The results obtained can be useful in providing specialized medical care for children of this age group, for optimizing the algorithms for preventive examinations and therapeutic interventions, and for offering the possibility of predicting the severity of the disease and timely surgical treatment of RSOC in the patients with inflammatory pathology of PNS.

Competing Interests

The authors declare that they have no competing interests.

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CASE REPORT

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Diagnosis of Intra-Abdominal Pressure in a Patient with Concomitant Injury as a Predictor of the Development of Acute Pancreatitis in the Post-traumatic Period: A Case Report

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Abstract

This article presents a clinical case of treatment of a patient with concomitant trauma. The increase in intra-abdominal pressure (IAP), as a predictor of the development of pancreatitis, was controlled by the developed method of radiation diagnostics. A method for assessing an increase in IAP, as a predictor of acute pancreatitis, is to quantify the volumetric blood flow in the superior mesenteric artery and superior mesenteric vein by Doppler ultrasound.(**International Journal of Biomedicine. 2021;11(3):305-307.**)

Key Words: acute pancreatitis • concomitant injury • intra-abdominal pressure • volumetric blood flow

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Abbreviations

AP, acute pancreatitis; **Hb**, hemoglobin; **IAP**, intra-abdominal pressure; **PRC**, pancreatitis risk coefficient; **RBC**, red blood cells; **SMA**, superior mesenteric artery; **SMV**, superior mesenteric vein; **WBC**, white blood cells.

Introduction

In patients with concomitant injuries, one of the complications is acute pancreatitis (AP), which develops both with trauma to the pancreas itself and without mechanical impact on it,[1] as a result of multiple organ failure.[2] In the post-traumatic period, one of the pathogenetic mechanisms of the development of AP is an increase in IAP, and, consequently, a decrease in abdominal perfusion pressure, which leads to the development of microcirculatory insufficiency and ischemia in cases of impaired blood supply to the pancreas, which has been noted in 36%-47% of patients in intensive care units. Thus, as a precursor to the development of AP in patients with concomitant injuries, it is necessary to control the state of IAP.[3]

A method for assessing the risk of developing AP in patients with increased IAP

Ultrasound diagnostics of the SMA and SMV is performed to determine the dynamics of the maximum *systolic* blood flow velocity (Vmax), the minimum diastolic blood flow velocity (Vmin) (only in the artery) and the volumetric blood flow (Vvol). In the case of increasing the above indicators of the SMA and inversely proportional decreasing them in the SMV, the pancreatitis risk coefficient (PRC)[4] is calculated by the formula:

PRC= Vvol SMA / Vvol SMV

Previously, this method was compared with the "Gold Standard" for determining IAP through the bladder.

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It is known that the following parameters are considered normal for the SMA: Vmax = 1.27±0.24 m/s, Vmin = 0.16±0.03 m/s, and Vvol = 791.82±48.47 ml/min. For the SMV: Vmax =0 .30±0.01 m/s and Vvol IMV=553.62±23.26 ml/min.[5]

Case Presentation

A 47-year-old man was admitted on 05.26.2020 to the Ulyanovsk Regional Clinical Center for Specialized Types of Medical Care named after V.I. EAT. Chuchkalov. Admitting diagnosis: Concomitant injuries. Closed spinal cord injury Contusion of the lumbar spinal cord. Closed unstable compression comminuted fracture of the body and arches of the L1 vertebra, grade 3. Closed compression fracture of the ThVI vertebral body, grade 1. Fracture of the spinous process of the ThV, ThVI vertebrae, the transverse process of the LIII vertebra on the left. Closed abdominal trauma. Rupture of the small intestine, mesentery of the small intestine. Hematomas of the mesentery of the small intestine. Hemoperitoneum. Contusions of soft tissues of the trunk, limbs. Traumatic shock, stage 3.

The man was injured as a result of a fall from a height. Complaints: pain in the lumbar spine, decreased sensitivity and motor activity in the lower extremities, lack of independent urination.

Clinical Findings and Diagnostic Assessment

Neurological status: clear consciousness, Glasgow Coma Scale - 14 points. Tendon reflexes D=S, from the legs were sharply reduced. Rough inferior paraparesis. Dysfunction of the pelvic organs by the type of urinary retention. There were no meningeal symptoms.

CBC: RBC - 4.18×10^{12}/L, Hb - 144g/l, Hct - 40.2%, WBC - 6.3×10^{9}/L, eosinophils- 2%, stab neutrophils - 6%, segmented neutrophils - 65%, Lymphocytes- 18%, monocytes -9%, ESR - 20 mm/hr.

Blood chemistry test (05.26.2020): total protein – 76 g/l, ALT- 65.4 U/L, AST-36.6 U/L, urea - 4.3 mmol/L, creatinine - 55.7 mmol/L, bilirubin - 6.5 mmol/L, amylase – 43 U/L, glucose- 5.1 mmol/L.

General urine analysis (05.30.2020): Normal values

CT scan of the brain and cervical spine (05.26.2020): No focal or bone-traumatic pathology was revealed. The midline structures of the brain were not displaced.

CT scan of the chest, abdomen, spine, and pelvis (05.26.2020): Closed unstable compression comminuted fracture of the body and arches of the L1 vertebra, grade 3. Closed compression fracture of the ThVI vertebral body, grade 1. Fracture of the spinous process of the ThV, ThVI vertebrae, the transverse process of the LIII vertebra on the left. There was blood in the abdominal cavity on the left.

IAP (05.26.2020) – 16 mmHg.

Ultrasound of the pancreas (05.26.2020): The pancreas was of a typical shape and location; the contours were clear and even. Head - 32 mm, body -18 mm, tail -30 mm. Echotexture was homogeneous and isoechoic. Wirsung's duct was not widened. SMA: Vmax - 1.36 m/s, Vmin - 0.18 m/s, and Vvol - 827.39 ml/min; SMV: Vmax - 0.28 m/s, Vvol - 568.2 ml/min

PRC=1.456.

Blood amylase (05.26.2020) - 96 U/L

IAP (05/28/2020) - 22 mmHg.

Ultrasound of the pancreas (05/28/2020): The pancreas was of a typical shape and location; the contours were unclear. Head - 35 mm, body - 21 mm, tail - 35 mm. Homogeneous echotexture, hyperechoic areas. Wirsung's duct was not widened. SMA: Vmax - 1.46 m/s, Vmin - 0.19 m/s, and Vvol - 891.35 ml/min. SMV: Vmax - 0.4 m/s, Vvol - 483.63 ml/min

PRC=1.843. Blood amylase (05/28/2020) -98 U/L

IAP (06.01.2020) – 25 mmHg.

Ultrasound of the pancreas (06.01.2020): The pancreas was of a typical shape and location; the contours were unclear. Head – 35 mm, body – 21 mm, tail – 35 mm. Homogeneous echotexture, hyperechoic areas. Wirsung's duct was not widened.

SMA: Vmax -1.74 m/s, Vmin - 0.21 m/s, and Vvol - 1008.72 ml/min; SMV: Vmax - 0.24 m/s, Vvol - 349.63 ml/min

PRC=2.885. Blood amylase (06.01.2020) - 96 U/L.

IAP (06.03.2020) – 26 mmHg.

Ultrasound of the pancreas (06.03.2020): The pancreas was of a typical shape and location; the contours were uneven, unclear. Head - 39 mm, body - 25 mm, tail -38 mm. Heterogeneous echotexture, areas of hyperechoicity. Wirsung's duct was expanded up to 4 mm. SMA: Vmax - 2.38 m/s, Vmin 0.23 m/s, and Vvol - 1609.01 ml/min. SMV: Vmax - 0.22 m/s, Vvol - 331.27 ml/min (Figure 1).

PRC=4.857. Blood amylase (06.03.2020) -148U/L

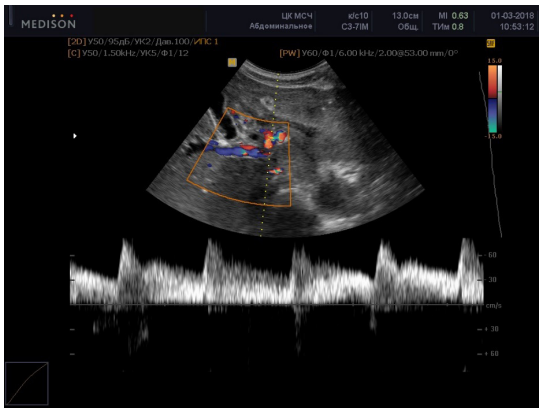

Fig. 1. *Ultrasound of the SMV in the color duplex scanning mode (06.03.2020)*

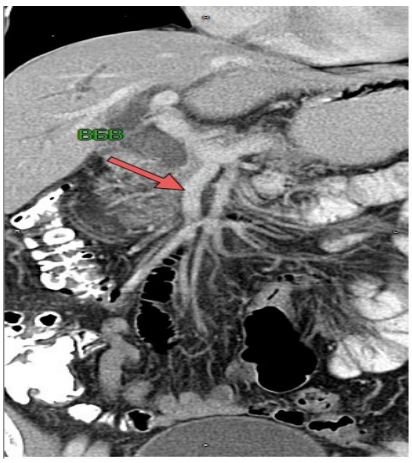

Fig. 2. *CT scan of the abdominal organs with 3D reconstructions.*

On Day 7 after admission, the patient was diagnosed with signs of AP. Starting from 06.01.2020, PRC>2. To control the data obtained, CT (03.06. 2020) of the abdominal organs with 3D reconstructions was performed (Figure 2). Expansion of the SMV was clearly visible. The patient was prescribed a standard treatment regimen for AP. Due to the timely diagnosis of intra-abdominal pressure and correction of therapy, the patient was discharged in satisfactory condition on Day 21 of treatment.

Competing Interests

The authors declare that they have no competing interests.

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CASE REPORT

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Evaluation of Vertical Guided Bone Regeneration Using a Particulate Form of Experimental Bioactive Glass in a Rabbit: A Case Report with Literature Review

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Abstract

***Background*:** There are a large number of different types of bone-grafting materials that are used for the regeneration of atrophied alveolar ridges in order to make dental implantation possible. However, available surgical techniques and materials for bone augmentation do not contribute to the achievement of the desired reliable results and require a search for new solutions to an existing problem. A group of synthetic osteoplastic materials based on bioactive glass (BAG) may become a matter of choice in bone tissue regeneration because of special osteogenic properties. The aim of this study was to visually and histologically evaluate the behavior of an experimental BAG in rabbit tibia bone samples, which were collected from the animal 6 weeks after filling the bone defects.

Methods and Results: The observation was carried out on one outbred rabbit whose tibia bone defects were filled with an experimental osteoplastic material based on the BAG. The chemical composition of the experimental osteoplastic material included SiO_2 (41%), Na_2O (21%), CaO (28.5%), P_2O_5 (6%), CaF_2 (1.5%), MgO (1%), Al_2O_3 (1%). For histological analysis, H&E staining of paraffin-embedded tissues was performed according to the standard technique. Light microscopy of tissue samples was performed using a Leitz HM-LUX microscope (Germany).

Six weeks after filling the bone defects, a strong bond between the augmented hard tissue and rabbit tibia was recognized. Also, a dense fusion of adjacent soft tissues with a newly formed bone without signs of chronic inflammation or graft particles in granular tissue was noted. Microscopic examination of the stained sections showed the presence of mature viable BT with a uniform distribution of osteocytes. Also, residual fragments of the degraded biomaterial surrounded by the fibers of a woven bone were revealed in several slices.

Conclusion: In accordance with the results of this experiment, it can be concluded that the usage of BAG related to the system SiO2(41%)-Na2O(21%)-CaO(28.5%)-P2O5(6%)-CaF2(1.5%)-MgO(1%)-Al2O3(1%) may increase the volume of bone without application of barrier membrane. However, further research involving more animals needs to be done to estimate the scientific significance of the obtained data and to evaluate the mechanical properties of augmented bone.**(International Journal of Biomedicine. 2021;11(3):308-314.)**

Key Words: bioactive glass • bone tissue • vertical guided bone regeneration • osteogenesis

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Abbreviations

BAG, bioactive glass; **BT**, bone tissue; **GBR**, guided bone regeneration; **DO**, distraction osteogenesis; **VGBR**, vertical GBR

Introduction

For the last few decades, mouth rehabilitation of patients with dental implants has become a widespread treatment modality in general practice. Such a rapid growth in this direction is caused by several factors, of which the most important and defining of the general tendency are the understanding and positive perception of patients who are considering dental implants as an effective and reliable treatment alternative for prosthetic replacement of missing teeth.[1,2]

In addition to patient comprehension, the growing number of field specialists also plays an important role in the development of dental implantology, because the outcome of implant therapy is highly dependent on correct digital treatment planning and effective cooperation of oral surgery and prosthodontics. Also, there are many other reasons related to the development and application of new materials, equipment, and technologies.[3,6]

However, despite the many positive aspects that promote the growth of dental rehabilitation through implants, the main obstacle to its spread is a deficiency of hard and soft tissues. In accordance with many studies, a sufficient amount of bone and attached gingiva around implants are the key prerequisites for their long service.[7,8]

In these particular cases, surgery for guided bone regeneration (GBR) is considered to be a viable opportunity for the patient and for the dental practitioner to place dental implants in an atrophied alveolar ridge. Data indicating the application of GBR first began to appear in the late 1980s. At that time, along with experimental studies in this direction, the usage of barrier membranes in clinical practice was going to be widespread.[9-11]

One of the most studied methods of surgical reconstruction of the alveolar ridge with barrier membranes is a two-stage surgery of horizontal augmentation. Data on the results of using this method has been published periodically for more than 30 years. For this period of time, every step of surgery had been optimized with consideration of possible complications, and successful outcomes of dental implant therapy were achieved.[12-14]

However, taking into account cosmetic aspects of patient rehabilitation with dental implants, the need for VGBR in esthetic zones also takes precedence. But unlike horizontal augmentation of buccal resorption of the alveolar ridge, reconstructing its vertical dimensions is the most difficult task.[15-17]

A systematic review of the main database and relevant articles from refereed journals for the period from 1980 to 2005 revealed that VGBR of a severely resorbed alveolar ridge is a sensitive surgical procedure. Also, it was noted that the normal functioning of dental implants placed in augmented regions is less dependent on the amount of regenerated bone and more related to the quantity and quality of residual hard tissues, which are responsible for the primary and secondary stability of a fixture.[18]

In some clinical cases, existing methods of vertical augmentation with different types of materials make it possible to obtain positive outcomes of reconstructive surgery. However, every chosen treatment protocol is characterized by insufficient reproducibility and a high probability of postoperative complications.[19,20]

The most commonly used method for vertical augmentation of the atrophied alveolar ridge, as well as with its horizontal reconstruction, remains GBR with the application of barrier membranes and particulate bone. However, as a dental practice has shown, to achieve satisfactory outcomes by using this technique, it is mandatory to use different types of titanium meshes or reinforced polytetrafluoroetylene membranes, but this can be a reason for wound dehiscence with subsequent infection, loss of graft material, and subsequent clinical failure.[21-23]

Using autogenous bone blocks for VGBR is not a widespread treatment modality because of a lot of shortcomings, such as an extended operative time, donor site morbidity and swelling, insufficient amount of graft and adjustment challenges, graft resorption, or absence of gradual vascular ingrowth.[24-27] As opposed to the surgery of autogenous bone harvesting, the practice of allogeneic bone blocks for the vertical reconstruction of the resorbed alveolar ridge is less invasive and might be promising, but still, they cause issues of disease transmission and immune rejection.[28-31]

A relatively new method for 3-dimensional augmentation of the resorbed alveolar ridge is the bone ring technique. This type of one-stage VGBR surgery involves placing a dental implant through preliminary adjusted autogenous or allogeneic bone block graft of the corresponding shape. However, in accordance with the results of several studies, the efficacy of this approach may be more significant in the reconstruction of a single vertical bone deficiency, but further observations are yet to be done.[32]

Alternative surgery of DO is associated with mounting a special device on the alveolar ridge, which helps to gradually distract a transport bone segment for new bone formation in the traction zone. Even though using this kind of VGBR allows achieving a maximum possible natural bone growth with simultaneous expansion of the soft tissues, the method of DO can be characterized as less predictable with a high probability of postoperative complications.[33,34]

Considering these methods, it is quite clear that available VGBR techniques and materials for bone augmentation do not contribute to achieving the desired reliable results and require the search for new solutions to an existing problem.

In order to assess the potential of new materials that could be applied with confidence for regeneration of BT, preliminary in vitro and in vivo studies with the application of clinically relevant models are of prime concern.[35]

It is well known that autogenous bone has osteoconductive, osteoinductive, and osteogenic properties. However, the harvesting of autograft is the reason for additional trauma, and the bone itself is subject to rapid resorption. Widely used bone substitute materials based on hydroxyapatite and tricalcium phosphate have no property to biologically bond with soft tissues and require the use of collagen membranes.[36,37]

In contrast, there is a new group of synthetic osteoplastic materials based on BAG, which possesses all the properties of an autogenous bone and can become a reliable alternative when planning a three-dimensional reconstruction of an atrophied alveolar ridge.[38]

The first melt-derived BAG was created thanks to the discovery of Larry Hench, who developed «Bioglass» based on oxides of silicon, sodium, calcium, and phosphorus. This material had the ability not only to mimic living BT, but also to induce the growth of a new bone.[39-42]

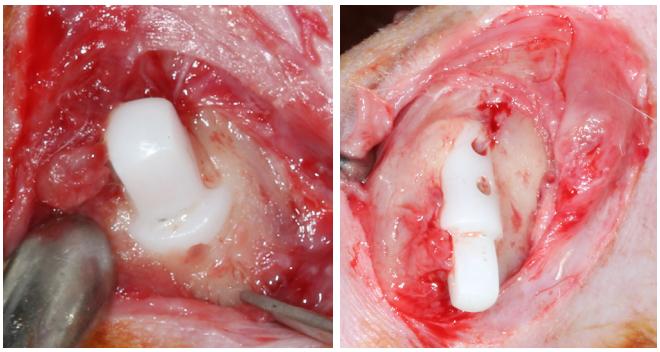
It should also be noted that over the years since the appearance of Bioglass 45S5, attempts are still being made to change its basic composition by adding some elements

to modulate the necessary properties and to improve its biocompatibility.[43-46]

Therefore, the aim of this study was to visually and histologically evaluate the behavior of an experimental BAG in rabbit tibia bone samples, which were collected from the animal 6 weeks after filling the bone defects.

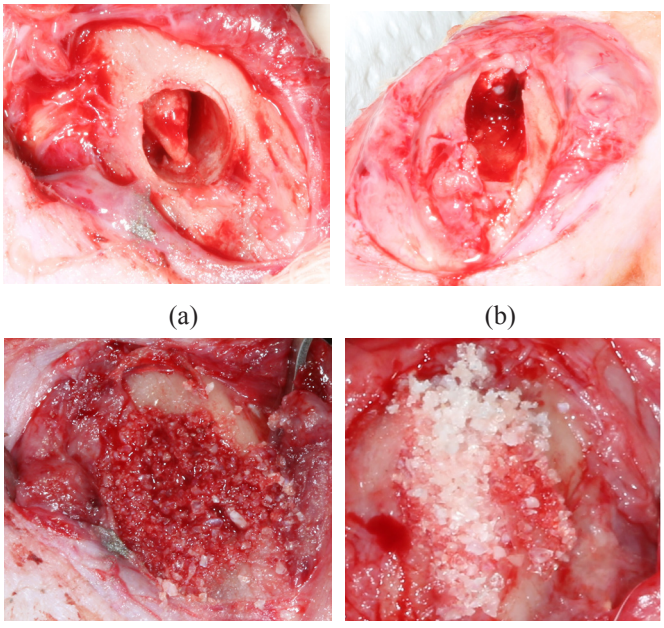
Material and Methods

In the present study, an observation was carried out on one outbred rabbit aged from 1.5 to 2 years and weighing 3.1kg, that was a participant in a parallel study, the purpose of which was the evaluation of osseointegration of hollow cylindrical zirconia implants placed in the rabbit tibia bones (Fig. 1 [a, b]). Because the animal was not sacrificed by the end of the time established for implant osseointegration, the bone defects (Fig. 2 [a, b]) caused by the removal of the implants were filled with an experimental osteoplastic material based on the BAG to assist the healing of bone (Fig. 2 [c,d]).



(a) (b)

Fig.1. *(a, b) Integrated hollow cylindrical zirconia implants (experimental) 6 weeks after implantation in rabbit tibia.*



(a) (b)

(c) (d)

Fig.2. *(a, b) Bone defects formed after removal of hollow cylindrical zirconia implants; (c, d) Bone defects filled with particulate BAG*

The experimental DAG was made traditionally by means of the melt-quenched method in a high-temperature furnace with silit rods at a maximum temperature of 1450 °C and hold-time for 1 hour, after which the molten mass was quenched into water. Formed granules of different sizes were ground in a ball mill and sieved in order to achieve particles with a size of 0.5-1 mm. The chemical composition of the experimental osteoplastic material included SiO_2 (41%), Na_2O (21%), CaO (28.5%), P_2O_5 (6%), CaF_2 (1.5%), MgO (1%), Al_2O_3 (1%).

The surgery was performed under general intravenous anesthesia using 1% sodium ethaminal solution in a dosage of 3ml/kg body weight, which was combined with local infiltration anesthesia with 2-4 ml of 2% lidocaine solution. Hair was removed in the areas of the planned intervention (area of the knee joints of both limbs) using scissors and a razor. After manipulation, the skin was treated with ethanol solution. The incision was made over the protruding part of the implant, which could be palpated under the skin. The length of the incision in each limb was about 4 centimeters. After elevation of the full-thickness flaps, the protruding part of the implant and part of the adjacent cortical bone were exposed (Fig. 1 [a, b]), and the implant was removed using a special instrument. Bone defects (Fig. 2 [a, b]) were filled in excess without the use of barrier membranes (Fig. 2 [c, d]). The amount of BAG material used in each site was around 1.5cm^3. The wound was closed with a single layer of sutures (Vicryl 5/0) (Fig. 3), after which the suture knots and surrounding skin were treated with a 5% alcohol solution of iodine.

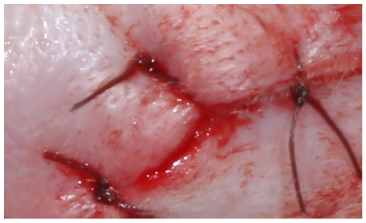
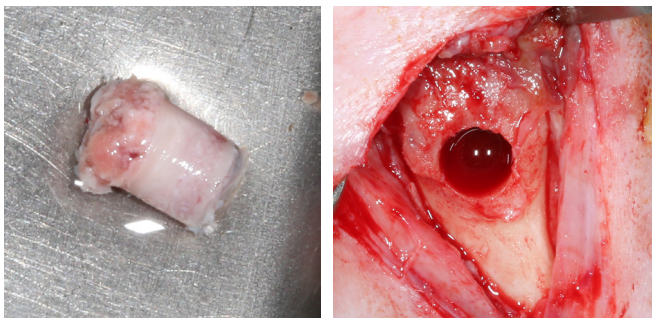


Fig.3. *Simple interrupted sutures.*

Reentry surgery was performed 6 weeks later. The operations were carried out according to a similar principle. Newly formed BT was collected using a trephine drill with an outer diameter of 5 mm at a speed of 1200 rpm under constant saline cooling (Fig. 4 [a,b]).



(a) (b)

Fig.4. *(a) A sample of a newly formed bone*
(b) Osteotomy after bone sampling with a trephine drill

Collected BT samples were fixed in a 10% neutral buffered formalin solution, after which they were decalcified, washed, dried, and embedded in paraffin. Ready paraffin blocks were subjected to sectioning, and slices of a 4-6μm thickness were obtained and stained with hematoxylin and eosin. Light microscopy of tissue samples was performed using a Leitz HM-LUX microscope (Germany).

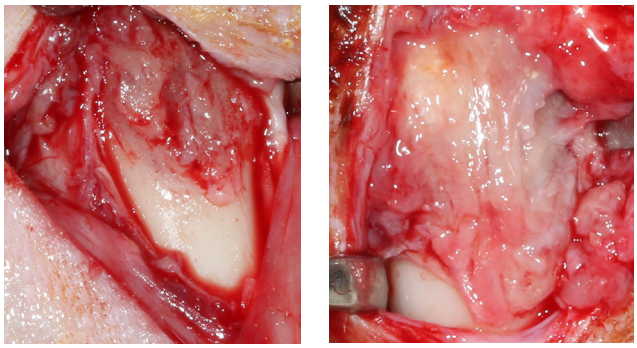
All experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals (The Institute of Laboratory Animal Resources, 1996). The study protocol was reviewed and approved by the Ethics Committee of Tashkent State Dental Institute.

Results

Before the surgery in the area of bone augmentation, the purpose of which was to identify the physiological response of soft and hard tissues to the implanted material, the health condition of the animal was assessed as normal. Visual examination of the limb skin showed normal healing without signs of purulent inflammation.

On palpation, regional lymph nodes were not enlarged. Also, in each limb, dense and immobile subcutaneous formations were present.

After making a longitudinal incision of the skin and periosteum over the protruding formations and elevating full-thickness flaps on the cortical surface of each tibia bone, an overgrowth of irregular size and shape was revealed, which differed from the natural bone in color and resembled a vitreous mass (Fig. 5 [a, b]). Also, when flaps were pushed away from the bone, a dense fusion of adjacent soft tissues with a newly formed bone without signs of chronic inflammation or graft particles in granular tissue was noted.

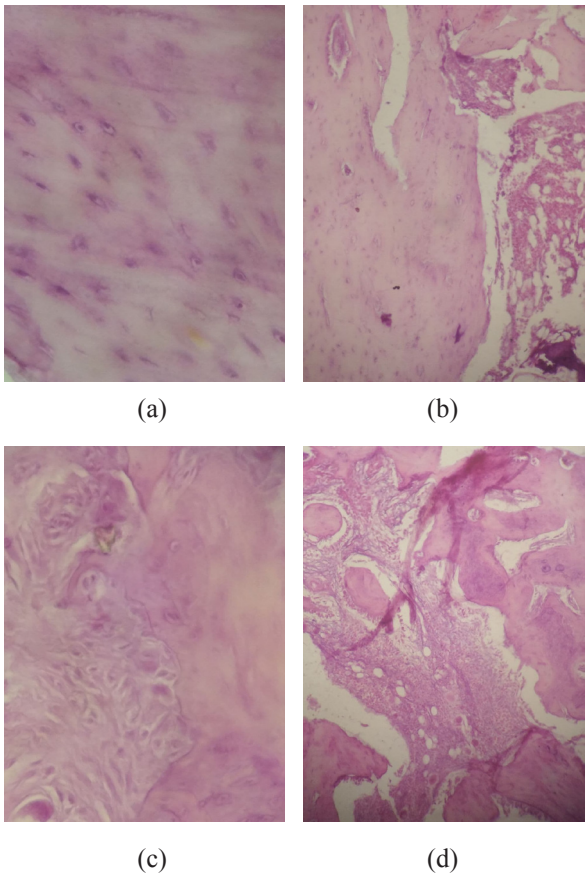


(a) (b)

Fig.5. *(a, b) Overgrowths of a newly formed bone in places of bone graft implantation.*

During the bone sampling, a strong bond between the augmented hard tissue and rabbit tibia was recognized. Also, there was no separation of augmented hard tissue from the host or occurrence of cracking lines along the visible interface, which could be the cause of a drilling vibration.

Microscopic examination of the stained sections showed the presence of mature viable BT with a uniform distribution of osteocytes (Fig. 6 [a, b]). Also, residual fragments of the degraded biomaterial surrounded by the fibers of a woven bone were revealed in several slices (Fig. 6 [c, d]).



(a) (b)

(c) (d)

Fig.6. *(a) Histological section of a bone collected from grafted site (H&E, ×20) – newly formed bone with uniformly distributed osteocytes; (b) Histological section of a bone collected from a grafted site (H&E, ×40) – non-mineralized osteoid matrix and newly formed bone; (c) Histological section of a bone collected from a grafted site (H&E, ×20) – woven bone surrounds residual fragments of BAG; (d) Histological section of a bone collected from a grafted site (H&E, ×40) – non-mineralized osteoid matrix at the interface with BAG*

Discussion

3D reconstruction of an atrophied alveolar ridge is not a fully resolved issue in dental implantology because existing methods of vertical bone augmentation are not without drawbacks.

Considering the priority of development and implementation of new bone grafts for bone regeneration, the experimental animal research with the application of a clinically relevant model is one of the first steps in evaluating their safety and efficacy.

The rabbit tibia model is a widely used one because it allows analyzing the biological response on bone graft material and dental implant. This circumstance had served as the basis for choosing this experimental model in this study.[47-49]

To date, there is a large amount of data indicating a high efficiency of bone-grafting materials based on bioactive glass, which is successfully used in filling bone defects after

removing benign neoplasms. Also, there are sources of data that confirm the positive behavior of this group of materials in the presence of pathogenic microflora.[50-54]

In the present experimental case study, a pronounced adhesion of a newly formed bone to the bone of the host was noted. Similar behavior of BAG bone grafts was observed in other studies, which confirmed the existence of its bonding ability to living tissues after the consolidation of the graft.[55-58]

Also, it should be noted that consolidation of the particulate bone graft and maturation of new BT occurred without using barrier membranes. This observation confirmed the ability of materials based on BAG to form a strong biological connection with soft tissues as well.[59]

Similar observations were made by other researchers, who did not find a significant difference between the usual grafting of bone defects using a BAG material and its application with a barrier membrane.[60]

Thus, in accordance with the results of this experiment, it can be concluded that the usage of BAG related to the system SiO_2(41%)-Na_2O(21%)-CaO(28.5%)-P_2O_5(6%)-CaF_2(1.5%)-MgO(1%)-Al_2O_3(1%) may increase the volume of bone without application of barrier membrane. However, further research involving more animals needs to be done to estimate the scientific significance of the obtained data and to evaluate the mechanical properties of augmented bone.

Competing Interests

The authors declare that they have no competing interests.

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BRIEF REVIEW

An Overview of Labor Pain: Components and Stages of the Labor Process

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Abstract

Labor pain (LP) is unbearable and a major source of anxiety and stress. Painful uterine contractions cause hyperventilation in the mother, and because of augmented catecholamine concentration, both the mother and her fetus will be hypoxic. Effective analgesia provides protection from difficulties and ensures good results in both the mother and fetus. Hence, the control of pain should form an integral part of labor management at any level. This brief review aims to identify LP and its effects on fetus and mother, stages of delivery and labor process, and components of LP. (**International Journal of Biomedicine. 2021;11(3):315-317**)

Key Words: labor pain • fetus • visceral pain • somatic pain • sensory fiber

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Labor is the complex and painful process through which a fetus and placenta are delivered from the uterus. The exact mechanisms triggering the onset of labor remain unknown. Some triggers of labor onset that have been suggested are an increase in myometrial OT receptor concentration, improved production of prostaglandin, and enlarged myometrial gap-junctions.[1]

Effects of labor pain on the fetus and mother

Pain is the most predominant signal of the beginning of labor. Labor pain is unbearable and a major source of anxiety and stress. Painful uterine contractions cause hyperventilation in the mother, and because of augmented catecholamine concentration, both the mother and her fetus will be hypoxic. Effective analgesia provides protection from difficulties and ensures good results in both the mother and fetus. Hence, the control of pain should form an integral part of labor management at any level. In addition, effective analgesia is crucial in some critical cases, as in women with cardiac problems and Grade II and Grade III dyspnea.[2,3]

LP by itself, aside from childbirth tissue injury, is directly or indirectly related to special influences on the mothers and fetuses. The response to the pain induces obvious stimuli to circulation and respiration, in addition to the function of neuroendocrine, especially in the hypothalamic autonomic centers and limbic structures, as well as in psychodynamic

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actions, such as apprehension and nervousness, which induce harmful consequences, which may occur to the mother and fetus. Appropriate pain relief is the only solution to get rid of and/or mitigate these responses.(4)

LP is one of the potent respiratory stimuli, causing an increase in ventilation and consumption of oxygen during contractions. Hyperventilation during contractions may cause temporary hypoxemia in the mother and, potentially, fetal hypoxemia. Respiratory depression results from analgesic procedures (eg, systemic opioid analgesia) and may exacerbate hyperventilation during labor. The hyperventilation causes acute respiratory alkalosis and leftward shifting of the mother's oxyhemoglobin degradation curve, reducing the transfer of oxygen to the fetus. Labor stress and pain also stimulate the sympathetic nervous system, leading to elevation of plasma catecholamine concentrations. Levels of norepinephrine and epinephrine are elevated during unmedicated labor (200%–600%) in addition to elevation of catecholamines during reduced uterine blood flow.(4,5)

Activation of sympathetic response by pain, induces cardiac output in a potentially harmful way in patients suffering from heart disease, anemia and preeclampsia. Additionally, it reduces gastric evacuation, which may lead to vomiting and nausea, and reduces the propulsive movement of the intestine, which may cause ileus and oliguria. The pituitary and placenta produce a great amount of β-endorphin to the blood but noticeably not enough to decrease pain successfully.(6)

Pain intensity and complaints related to vaginal delivery and labor differ extensively in parturients. The pain rating index (PRI) is lower in parous mothers than nulliparous mothers. In addition, an important variance between parous and nulliparous in pain sensory quality has been detected. The scores of pain vary from mild to severe (Figure 1); the PRI in women during labor is commonly eight to ten times greater than in those patients suffering from post-herpetic neuralgia, phantom limb pain and cancer pain.(4,5)

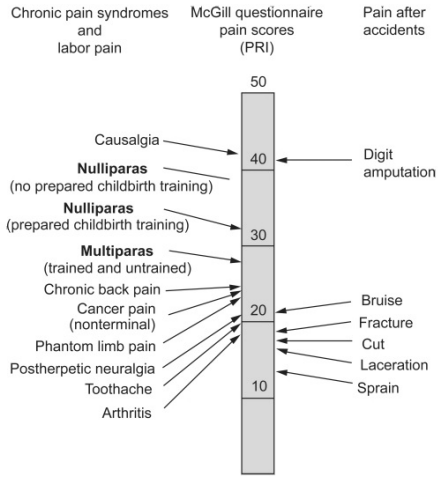

Fig. 1. *Pain intensity according to the McGill Pain Questionnaire.(1)*

Finally, severe mental health impairment may result from continuous severe pain, which affects the maternal–neonatal connection and sexual relationships, and may lead to post-partum depressive disorder, and, rarely, to post-traumatic stress disorder.(4)

Sensory Nerve Supply of the Birth Canal

Sensory fibers from the uterus pass by sympathetic nerves through the inferior hypogastric plexus (T10–L1) and from the cervix by parasympathetic nerves (S2–4). The pudendal nerve (S2-4) supplies vaginal and pelvic outlets. Minor supplies are also derived from the perineal branch of the posterior (L2-4) and genito-femoral nerves. (L1, 2) (Figure 2).(7)

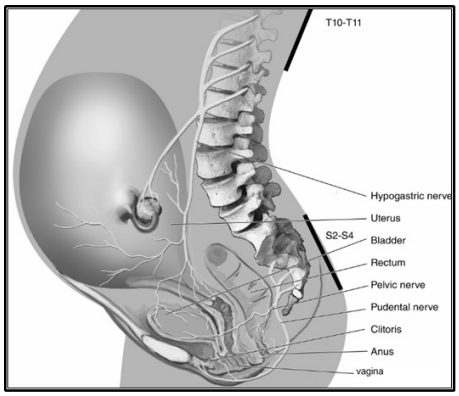

Fig. 2. *Pain pathways in a parturient* (7)

Stages of labor and delivery process

The labor and delivery process includes three important stages as detailed below:(1)

Stage I begins with frequent uterus contractions (frequent uterine contractions with gradual cervix dilation) and ends with full cervix dilation.

Stage II begins with full cervix dilatation and continues until infant delivery.

Stage III begins with infant delivery and continues until complete expulsion of the placenta and membranes.

Components of labor pain

LP is divided into two kinds, somatic and visceral. In Stages I and II of labor, the cervix has a great role in normal labor. During the early Stages I and II of childbirth, the visceral LP is manifested. The cervix is stretched by pressure during uterine contractions; the lower segment of the uterus is thinned out, stimulating excited nociceptive sensory neurons that innervate the endo-cervix and distal segments (T10-L1).(8)

Somatic pain occurs at the end of Stage I and during Stage II. It results from sensory nerve endings, which innervate the surface of vagina, perineum, and cervix, and happens due to distension, stretching, ischemia and stretching of vagina, perineum, and pelvic floor. Regular and rhythmic uterine contractions are more forceful during the active stage through the descent of the fetus. With more dilation of the vaginal cervix, the severity of labor pain increases directly with more frequency, duration, and intensity of uterine contractions.(8,9)

The LP manifests during Stage I as visceral pain. Visceral pain is transmitted by small unmyelinated 'C' fibres which travel with sympathetic fibres and pass through the uterine, cervical and hypogastric nerve plexuses into the main sympathetic chain.(8) The nociceptors of the sympathetic chains pass into the white ramus related to spinal nerves of

T10-L1 and enter through the dorsal nerve root to the dorsal horn cells of the spinal cord. At the level of caudal and rostral extension with dorsal horn cells, some fibres cross over, which leads to poorly localized pain.[8] LP stage I has been shown to be a pain referred to the anterior abdominal wall and to the back (Figure 3) as the anterior abdomen and low back areas are supplied by the same spinal roots that are receiving pain impulses from the uterus. This pain is dull in character.[1,10]

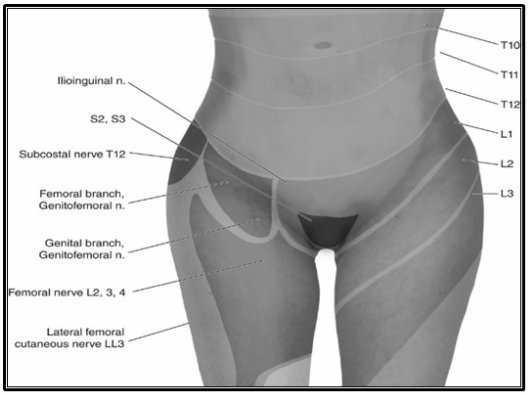

Fig. 3. *Dermatome levels of the thigh, hips, perineal area, and lower abdomen.* [7]

Pain during Stage II labor follows a different pathway from labor Stage I. It has been demonstrated to be somatic pain and is diffused by smooth, myelinated, fast conducting A-delta fibers. Diffusion is carried out through the perineal branches of the posterior cutaneous nerve of the thigh and pudendal nerves to S2-4 nerve derivations. From genitofemoral and ilioinguinal nerve roots through cutaneous branches, somatic fibres carry sensory nerve fibers to L1&2. The somatic pain comes close to labor, is intensive, and definitely localized to the rectum, perineum, and vagina, which radiate to T10 and L1 dermatomes with more resistance to opioid medications than visceral pain.[8,11]

It is important to realize that these two types of sensation are not mutually exclusive: pain associated with labor Stage I does not stop miraculously with the entry into labor Stage II, but it is often superseded by pain resulting from distention of the perineum due to the descent of the fetus's head.[12]

Briefly, all somatic and visceral nerve impulses pass into the spinal cord through dorsal horn cells, which have been handled and transferred to the spinothalamic tract, and travel to the brain in an orderly manner. Transmission of the impulses to limbic and hypothalamic systems determines the autonomic and emotional responses related to pain.

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Competing Interests

The authors declare that they have no competing interests.

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POINT OF VIEW

Adaptive Changes in the Psyche of Homo Sapiens during the Period of the Singularity (Part 3)

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Abstract

Personal constitutional and acquired predispositions form preferences in the vectors of perception of information (cultural) sentences of the environment. On these vectors, contextual factors are formed that affect the processing of incoming information, the formation of representations and images, which determine the interpretation of lexical signs. Multiplication of contexts creates metacontexts that define the boundaries of virtual reality. One of the design features of Clip thinking (ClipT) is the formation of metacontexts by external structures: network associations. The metacontexts of ClipT form a new structure of communicative experience that changes the self-identification and socialization of the subject, causing a state of dependence. ClipT has significant similarities with hieroglyphic thinking (HieT), which consists of quantized combinations: image + sense + emotion + tone. Hieroglyphics (thinking and writing) have a metacontext nature, being a formation of an ethnic scale. According to some structural parameters, ClipT and HieT are similar to the level of identity. Fundamental factors are the external genesis of metacontexts; the imagery of thinking; elimination of causal relationships and abstraction; visual "receiving-transmission" of the sense of the perceptual image, including the graphic image; rigidity of mental and behavioral structures; embedded experience; etc.

The integral structure of a legitimizing nature (HieT + writing + language + metacontext continuum [ethnocultural matrix]) forms a parametrically conjugated social structure derived from it. A monolithic socio-cultural conglomeration with a self-reproduction mechanism is created. We believe this principle is universal, with the possibility of extrapolation to any socio-cultural structure.

Currently, the HS population is in a state of forming a universal cultural matrix with the potential to replace ethnocultural matrices. ClipT—the new operating system of the psyche—defines and unifies the transformation of ethnocultural matrices with the vector of universalization. The significant similarity of hieroglyphics with the parameters of ClipT contains the extrapolation potential of modeling (sociological and mathematical) the expected ethnic/universal dynamics of conglomeration: ClipT (psyche as a whole) and congruent social construction. The identity points of ClipT and HieT can find application in the constructions and artificial intelligence learning, as reference points of operating systems of thinking and language, in the structure of which there are no algorithms for cause-effect relationships, analysis, feedback, abstract thinking, and classification structures dominate. (**International Journal of Biomedicine. 2021;11(3):318-322.**)

Key Words: hieroglyphic thinking • clip thinking • metacontext • artificial intelligence • ethnocultural matrix

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Abbreviations

AI, artificial intelligence; **ClipT,** clip thinking; **ConceptT**, conceptual thinking; **EE**, embedded experience; **HS**, Homo sapiens; **HieT**, hieroglyphic thinking; **IU**, information universe.

Basic Part

Imaginations of the HS psyche create and structure the information field of the two-component (real/virtual) environment. The virtual component made it possible for HS to evaluate an object outside the "reactive behavior" paradigm. The real reflected object in the variants of future interaction (hunting, protection, rituals, etc.) was transformed into a virtual one, with the possibility of variable processing of the methods of interaction and the search for optimal tactics of behavior. Variants of behavior are formed by the interpretation of a set of representations, "extraction" from subjective

experience, which determines the variant of behavior.[1] The totality of "imaginations" (perceptual, mental), filled with the content (meaning) of images, forms psychological constructs and synthesizes their connections, constructing an integral two-component environment.[2,3]

The dynamics of the HS development transformed the initial thinking from "concrete-objective" to "visual-figurative," then to "abstract-logical." In other words, from thinking based on interaction with a real object to the possibility of establishing relationships between objects (close forecast), then to operating with categories that are absent in the real sector of the environment. The perception and processing of images of the external environment formed ideas, symbols (image+sense), which initially had a single storyline, a context that integrated increasingly complex ideas, mental constructs. We deliberately simplify this construction, without bringing the hierarchy of HS needs, to build a general scheme for the integration of images, motivation, thinking, verbalization and behavior.

During the 50,000-year period, HS has been forming a new (two-component) habitat for hominids, in which the HS thinking (psyche as a whole) was the tool, creating plot-related sequences of mental constructs. Mental activity is the operation of "signs" and the construction of "sign" models of reality by the psyche. Real objects and their reflections ("signs") are inextricably linked. Artificial "signs" (words, mathematical signs, etc.) are not associated with the substituted natural objects. The two cerebral hemispheres of the human brain are characterized by different cognitive processing modes. As known, tonal information (melody and intonation) is processed in the right hemisphere, logical-verbal information - in the left hemisphere. The left hemisphere operates with artificial "signs," the right with natural ones.[4]

Book printing, which has shaped "textual" thinking, has created a new type of thinking—ConcepT. The mental, social, and technological dynamics of the 18-20th centuries was formed on the basis of the ConcepT.

The operational basis of ConcepT is the construction of complete sequences based on causal relationships, the interference and integration of which forms systemic formations (constellations) in all areas of the HS activity.

We believe that an important feature of these constellations is the possibility of synthetic unions and analytical (critical) assessment of dynamics and results. Causal relationships, semantic unity, logical sequence, as a constructive tool of ConcepT, make it possible to classify, evaluate, synthesize and analyze each stage of the formation and dynamics of these constellations. In other words, the presence of feedback, analytical (critical) assessment makes it possible to correct and change the vector of development of both mental and behavioral structures. A significant limitation, a sign of insufficiency in modern conditions is the failure of ConcepT in critical situations with a rapid increase in information, leading to "transcendental inhibition."[5]

The modern dynamics of technological development transform HS thinking. Communication technologies change the cultural matrix, with a significant delay in reflection.[6] Changes in thinking algorithms (ClipT), under the regulatory influence of interaction with the "information universe" (IU),[3] led to the elimination of the mechanism of autonomous construction of images that have a semantic sequence. The result is in a decrease (elimination in the future) of the ability and needs to build cause-and-effect relationships, with the replacement of interdependence (correlation) with an algorithm; a reduction of synthetic and analytical (critical) functions and generalizations; the loss of feedback, insensitivity to contradictions; a decrease in vocabulary (linguistic minimalism). Structural formations of ClipT codes (meme, gif, smile-emoticon, etc.) are nonlinear and hybrid (the combination of verbal, visual, and other components makes them equivalent). In other words, the "new literacy" of ClipT differs in the principles of coding from the "text" ConceptT. ClipT forms and embeds sequences that do not have complete semantic unity, semantic links and do not involve reflection. The predominance of visual discrete information, which has a higher speed (relative to semantic) processing and does not have emotional derivatives, forms the ability to "multitask," speeds up and shortens psychological (subjective) time. ClipT is discrete, not integrated into the reflection of life and forms a general "picture of the world" as a fragmentary mosaic with reduced emotional background.

We believe that the new operating system of the psyche (ClipT) is formed in accordance with and depending on changes in the main parameters of the virtual sector of the environment—the information universe (IU). The dynamics of constructive change in thinking (psyche) can be calculated based on the need (perspective) of a conjugate combination of the main parameters of interacting systems: psyche (ClipT) and IU.

The standardization of the virtual component of the habitat forms communities united by universal communication codes, symbols, rituals adopted by these communities. Total "particulars" destroy centralized norms and ethnic standards.

Personal constitutional and acquired predispositions form preferences in the development of the perception vectors of informational (cultural) offers of the environment. We believe, understanding the magnitude of the simplification, that it is on these vectors that contextual factors are formed. These factors affect the system of processing incoming information, constituting an actual situational context, forming imaginations. The context that determines the interpretation of linguistic signs is a set of factors of the environment of the object's existence, in addition to the sign itself,[7,8] the cognitive structure (frame) of schematization of experience.[9] Frame constitutes the structural context of interaction,[10] the scheme of images.[11]

The sequence of the interaction of contexts (frames) leads to "layering," multiplication of contexts, creating the ultimate metacontext and defining the boundaries of social (virtual) reality.[10] It must be emphasized that exactly the metacontexts constituting the sense of our experience, not the ontological structure of objects, constitute reality.[12]

We believe that in the structure of ClipT, each perceived image (each structural unit) includes an associated (but not actualized) context. Due to the lack of cause-and-effect relationships of the perceived images, this construction does not have a "metacontext" covering contexts of the same

logical type. The structure and parameters of the actual metacontext in ClipT are formed, imposed, and updated by network associations, which form (regardless of scale) closed systems with their own identification codes. In other words, the constructive feature of ClipT is the formation of the metacontextual potential under the regulatory influence of external systemic formations: network associations that have unified cultural symbols, their own dialect, providing equal access and a way of formatting information. The result is the formation of a new structure of communicative experience, leading to changes in the socialization of the subject, deformation of self-identification, atomization of society, and dependence on an impersonal network association. Replacing the algorithm for creating authentic images with the implantation of generated images of external genesis, forms the embedded experience (EE). That is, a subjective experience is formed, the basic arsenal of which (embedded image + sense) is outside the scope of personal experience, supplementing and replacing the integral structure of individual knowledge and skills acquired during training. Thinking algorithms, built on the basis of EE, actualize the potential of creativity and the creation of abstract-logical constructions based on structures from the arsenal of EE. The defining vector of thinking is the appeal to the EE, which determines the options for behavior in ClipT.

From the basic cognitive ability of subject construction, cognitive abilities associated with languages of different types were formed in phylogenesis.[13] The native "proto-languages" of primitive people did not have an analytically differentiated structure.[14] Thinking always adapts to the peculiarities of the language, as a factor in the socio-cultural environment.

ClipT, being a new operating system of the psyche that adapts HS to changes in the virtual segment of the environment, in our opinion, has a significant similarity with HieT. Of all the pre-existing hieroglyphic types of writing and thinking, we believe that the most suitable for comparison and research is the classical (not pinyin) hieroglyphic thinking and writing in China.

Hieroglyphs arose from pictographic writing transmit the general idea (sense) of the designated object, phenomenon, concept, and include an image and emotion, being a structural unit of HieT. HieT (a 5000-year period of existence), consisting of quantized integral combinations (image + sense + emotion + tone) is semantically discrete, coming closer to the thinking of the Neolithic people. The Chinese language genetically recorded the earliest form of thinking – figurative thinking.[15]

Hieroglyphic writing limits the development of language and eliminates the space of abstract thinking.[16] The perception of the world through hieroglyphics is consistently progressive, not divided by signs.[17] In colloquial Chinese, the context is significantly limited in accordance with the algorithm for selecting the optimal meaning of a word from a variety of options. This limitation makes the lexical potential several times higher in comparison with the spoken one, increasing the role of the metacontext to the size of a directive factor. The metacontextual nature of hieroglyphic writing is an ethnic-scale formation that structures and determines communication of a legitimizing nature. It is the metacontexts that form not only the stability of the integral structure (hieroglyphic writing, thinking, and language), but also the balancing of society as a whole (for example, the Confucian category of ritual and ethics - "Li"), ensuring the replication of standard mandatory forms of behavior, forming the EE. The "space of imagination" in the Chinese tradition, in contrast to visual perception (limited by the limits of visibility), is infinite. A lot of events and actions, separated in time and space, are transmitted at a time. In this coordinate system, HS is not the "center of the universe," but an element, a participant. We believe that the figurative illustration is the "parallel perspective" of Chinese painting, reflecting the dualism of the Chinese worldview.[18]

Being a derivative of a pictorial image, in contrast to phonetic writing, hieroglyphs reproduces a complex of associative imaginations associated with the hieroglyphic "image of a concept," and denotes the sense of a perceptual image through a graphic symbol. When reading hieroglyphs, understanding the meaning occurs in a holistic manner, instantly. Alphabetic writing (ConceptT), to reveal the meaning, requires reading all letters, with the subsequent construction of an image and filling it with sense, sequential processing ("step by step"). Hieroglyph contains "sense" only as an integral image, and the analytical division into graphemes does not reveal the meaning of the integral hieroglyph.

Hieroglyphic writing provides immunity to any external influence, eliminating abstractness during translation, radically changing the sense. Any alien system of representations and meanings is leveled and assimilated by specific symbolic graphemes of hieroglyphic writing and language.[19]

We believe that the fundamental constructive similarity between ClipT and HieT is the formation and actualization of the metacontext under the regulatory influence of external factors. In many structural parameters, ClipT and HieT are similar to the level of identity. Here are some parameters: 1) the dominant flow of information is visual, with a similar unit of coding (image-meaning-emotion), including a graphic image; 2) semantic discreteness; 3) sequential-progressive perception, not separated by signs; 4) simultaneous perception of heterogeneous information; 5) the universal codified communication system, cross-border to interdisciplinary borders; 6) elimination of causal relationships; 7) lack of analytical and synthetic functions; 8) hybridity of structural codes (equivalence of components); 9) one-pointedness in thinking and difficulties in correction; 10) a feedback loss, as a result of the lack of analysis and signs of separation (perhaps this is the root cause of the appearance of "parallel perspective" as a self-consistent picture of the universe); 11) the rigidity of the dynamics of the vectors of development of mental and behavioral structures, etc.

HieT excluded the creation of abstract thinking as a mechanism of "distraction" to highlight essential features. In the naturalistic world of the Chinese, classification structures dominate, instead of abstract logical ones (numerology instead of logic).[20] We believe that the listed points of the ClipT/HieT identity can find application in programming and AI learning, by extrapolating the principles of operating systems of thinking and language, in the structure of which there are no algorithms of cause-and-effect relationships, analysis, abstract thinking, and classification structures dominate.

Language, as a system of reflection, fixes in its structure the specifics of ethnically conditioned thought.[21] Thinking and language (writing, phonetics, grammar, etc.) are part of a culture that has a selective effect on the population.[13,22] Culture as a whole is a "grid" that structures the evaluation criteria of environmental images,[23] forming ethnocultural matrix.

We believe that the integral structure (Hie-writing + Hie-thinking + language + continuum of metacontexts (Ethnocultural matrix)) is an integral part of a single, holistic education, including a parametrically conjugate, congruent construction of a social structure (hierarchical, centripetal, regardless of the name). We consider that primary in this structure is the "cultural matrix" (language, writing, thinking, metacontext), which has existed unchanged for about 5000 years, while the stable, unchanging social component associated with the matrix (documented statehood) has existed for about 3500 years. We believe that the superimposition of the cultural matrix on the associated social structures derived from it forms the stability and self-reproduction of the social structure as a whole, regardless of external influences.[24] In other words, the ethnocultural matrix forms and stabilizes its derivative, parametrically conjugated structure of the social structure (statehood), in accordance with ethnic characteristics. We believe that this principle is universal, with the possibility of extrapolation to any developed socio-cultural structure.

Currently, a global system of unified coding marks is being formed, including identification systems of social networks, the transformation of evaluation criteria, letters and speech, neologisms, emoticons (containing "image + sense + emotion") and other signs, codes, methods of communication and perception. The expected milestone result of these changes is the formation of a universal coding system of global communication, universal metacontexts, synergistic (multiparadigmatic) thinking, transboundary in relation to disciplinary boundaries.[25]

ClipT, formed by the psyche for adequate interaction with the information field of the IU, contains signs and properties that constructively coincide with HieT. As an attractor of the dynamics of the observed changes in the HS psyche, we consider the expected integral structure of conjugated constructs (the "information universe" and the adapted psyche of HS), in which ClipT is the fixed part of the transformation of the psyche. A feature of this construction is the inversion of the vector of the formatting influence: from the "information universe" in the direction of the HS psyche. The interference of the "information universe" and ClipT (psyche as a whole) determines and unifies the transformation vector of ethnocultural matrices. Currently, the HS species population is in a state of forming a universal cultural matrix with the potential to replace ethnocultural matrices.

Conclusion

The legitimizing constructs of classical Hieroglyphics (thinking, writing, language, metacontext), making up the ethnocultural matrix, form a parametrically coupled stable social structure. The expected result of modern changes in the HS psyche (ClipT, the formation of a coding system of global communication and universal metacontexts, as well as a synergetic thinking, cross-border to disciplinary boundaries) is the creation of a universal cultural matrix with the potential to replace ethnocultural matrices. The constructive similarity of Hieroglyphics with ClipT contains the potential for sociological and mathematical modeling of the expected dynamics of conglomeration: ClipT (the psyche as a whole) and a social structure congruent to changes in the HS psyche.

Competing Interests

The authors declare that they have no competing interests.

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REVIEW ARTICLE

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Milestones in Molecular Mechanisms of Adipogenesis and Adipose Tissue Plasticity

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Abstract

This review focuses on the problem of adipogenesis mechanisms and the biological role of adipose tissue (AT) in the human body. Over the past decades, various types of adipocytes have been identified and characterized—white, brown, beige, yellow, and pink. An important feature of AT is a high plasticity and the ability to transdifferentiate and de-differentiate into another cell type. In this case, the pathway of transformation mostly depends on adipocytes' cellular and metabolic microenvironment. The mechanisms of adipogenesis and the ways of its regulation remain not fully understood. The principal role in the terminal differentiation of preadipocytes is assigned to PPARγ and receptors activated by bone morphogenetic proteins, insulin, and cortisol. However, in chronic inflammation, adipogenesis is suppressed and old adipocytes increase the production of proinflammatory cytokines, which leads to the death of inflamed cells and hypertrophy of neighboring adipocytes. Thus, disruption of adipogenesis, premature aging of white adipocytes, perturbations in the metabolic and cellular microenvironment of preadipocytes, and early apoptosis of fat cells cause the development of insulin resistance and metabolically unhealthy obesity.(**International Journal of Biomedicine. 2021;11(3):323-332.**)

Key Words: adipose tissue • adipocytes • adipogenesis • transdifferentiation

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Abbreviations

AT, adipose tissue; **BM**, bone marrow; **BMPs**, bone morphogenetic proteins; **BMAT**, bone marrow adipose tissue; **BMAC**, bone marrow adipocytes; **BAT**, brown adipose tissue; **BeAC**, beige adipocytes; **BrAC**, brown adipocytes; **BMI**, body mass index; **C/EBPs**, CCAAT-enhancer binding proteins; **FAs**, fatty acids; **FAHFAs**, fatty acid esters of hydroxy FAs; **HIF-1α**, hypoxia-inducible factor-1α; **LDLR**, low-density lipoprotein receptor; **MCSs**, mesenchymal stem cells; **Myf5**, myogenic factor 5; **PDGFRα**, platelet-derived growth factor receptor α; **PPARγ**, peroxisome proliferator-activated receptor γ; **ROS**, reactive oxygen species; **WAT**, white adipose tissue; **WAC**, white adipocytes.

Introduction

Currently, we are facing a significant revision of understanding the biological role of adipose tissue (AT) in the human body. Over the past decades, various types of adipocytes have been identified and characterized—white, brown, beige, yellow, and pink. AT possesses a high degree of heterogeneity even within the same fat depot. An important feature of AT is a high plasticity and the ability to transdifferentiate and de-differentiate into another cell type. In this case, the pathway

of transformation mostly depends on adipocytes' cellular and metabolic microenvironment. Preadipocytes are formed from different progenitor cells: for instance, thermogenic adipocytes may have myogenic (like brown cell type) or adipogenic (like beige cell type) origin. The mechanisms of adipogenesis and the ways of its regulation remain not fully understood. The principal role in the terminal differentiation of preadipocytes is assigned to PPARγ and receptors activated by bone morphogenetic proteins, insulin, and cortisol. However, in chronic inflammation, adipogenesis is suppressed and old adipocytes increase the production of proinflammatory cytokines, which leads to the death of inflamed cells and hypertrophy of neighboring adipocytes. Thus, disruption of adipogenesis, premature aging of white adipocytes, perturbations in the metabolic and cellular microenvironment of preadipocytes, and early apoptosis of fat cells cause the development of insulin resistance and metabolically unhealthy obesity.

Types of adipocytes

AT is divided by localization and cell morphology into four main types:[1] white, brown, beige,[2] and yellow. The last type was identified in the bone marrow (BM).[3] White adipose tissue (WAT) is characterized by heterogeneous localization and cellular composition.[2] WAT is divided into visceral and non-visceral, which can be subdivided into subcutaneous and intradermal.[4] Intradermal AT is involved in wound healing and hair development,[2] whereas the main functions of subcutaneous AT are energy storing and hormone production.[4] WAT consists of different cell types: white adipocytes (WAC), white preadipocytes, mesenchymal stem cells (MSCs), pericytes, monocytes, and macrophages.[2] Visceral WAT differs from subcutaneous fat in cellular composition, increased resistin production, lower leptin secretion level[5] and lower insulin sensitivity.[6] Therefore, the accumulation of visceral fat is associated with the development of metabolic syndrome,[2] type 2 diabetes, cardiovascular failure, and fatty liver disease.[7] Furthermore, visceral AT exhibits apparent metabolic differences: the rate of lipolysis and formation of free fatty acids (FAs) is significantly higher in visceral than in subcutaneous depot.[5] It should be mentioned that storing fat on the thighs and trunk significantly reduces the risk of metabolic syndrome,[6] and a high secretion of leptin prevents the development of neurodegenerative diseases.[5] The loss of WAC in subcutaneous limb fat depots takes place in cases of lipodystrophic diseases, such as familial partial lipodystrophy and lipodystrophy caused by antiretroviral therapy in HIV-positive patients. This process is associated with simultaneous accumulation of visceral fat, including the areas of brown adipose tissue (BAT) localization.[8,9] Hypertrophy of beige (induced brown) AT and its replacement with WAT in the dorso-cervical region is observed in HIV-positive patients.[10] In these patients, lipodystrophy is paradoxically associated with metabolic signs of obesity—insulin resistance, hyperglycemia, hepatosteatosis, hypertension and dyslipidemia.[11,12] Thus, deficiency of AT, as well as obesity, is associated with severe metabolic disorders.

BAT is localized in cervical, axillary, paravertebral and supraclavicular areas. The main functions of brown adipocytes (BrAC) are adaptive thermogenesis[13] and endocrine regulation of lipogenesis and adipogenesis.[14] The number of BrAC decreases during aging. According to Rui et al., [13] it may be evolutionary associated with human behavioral adaptation— clothes wearing.

According to the experimental data, a significant decrease in the number of BrAC in mice is associated with aging.[15] Simultaneously, as the number of WAC increases, the transdifferentiation of BAT to WAT intensifies. The same results were presented in a study of biopsy samples of pericarotid cervical fat depots in humans.[16,17] It should be mentioned that the complete absence of BAT in the body leads to fatal hypothermia and death in mice.[18]

Bone marrow adipose tissue (BMAT) is a separate depot of adipocytes in the human body.[3] It consists of constitutive fat tissue localized in the distal bones of the skeleton, as well as adjustable AT diffusely scattered in the spine and proximal limb bones and is responsible for interaction with environmental factors.[19] BMAT plays an important role in bone metabolism and the regulation of osteoblast activity.[20] Unlike BrAC and BeAC, BMAT does not express UCP-1.[19] Reactions to adrenergic stimulation are expressed to a lesser extent in adipocytes of the BM than in white ones.[21] Such resistance of yellow adipocytes manifests to a greater extent in the distal regions of the skeleton, where adipocytes have larger size and constitutive phenotypes. It is believed that adrenergic sensitivity of adipocytes is influenced by the cellular microenvironment. Importantly, women are more susceptible than men to lipid-droplet remodeling of yellow adipocytes.[21] During fasting, bone marrow adipocytes (BMAC) do not serve as a fuel source and thus become the main provider of the circulating hormone adiponectin.[20]

Adipocytes heterogeneity

Adipocytes could be considered as quite heterogeneous cells. The same depot cells may be characterized by different functions. The WAT preadipocytes with a low CD9 expression level have higher adipogenic potential, whereas preadipocytes with a high CD9 expression level mostly display profibrotic and proinflammatory properties.[22] Based on single-cell transcriptome profiling, WAC could be divided into four subtypes, including beige thermogenic adipocytes and a subtype specialized in leptin secretion.[23]

BrAC differ in the thermogenic potential. Low thermogenic adipocytes have a decreased UCP-1 and adiponectin expression, and they contain larger fat droplets and fewer mitochondria.[24] There are two BeAC types: ordinary BeAC and glycolytic BeAC (g-BeAC).[18] g-BeAC control thermogenesis and glucose homeostasis in the absence of β-adrenergic stimulation. According to Chen et al.,[18] heat stress may induce g-BeAC formation from white preadipocytes.[18] The ratio of white, beige and brown adipocytes is unstable and may differ[25,26] in various fat depots, depending on different environmental factors, such as stress, cold stimulation, diet, and lactation. Due to the ability of adipocytes to differentiate and de-differentiate into another cell type, the cell number and phenotype incidence may be variable in different fat depots.[27] Figure 1 illustrates the

development paths of different adipocyte types and subtypes. Myf5- MSCs are progenitors of WAC, which are able to differentiate into pink and beige adipocytes. Myf5+ MSCs are BrAC progenitors. The ultrastructural difference is shown (the lipid-droplet–nucleus-mitochondria ratio). Thermogenic adipocytes are reached by mitochondria, whereas fat storing cells are reached by lipid droplets.

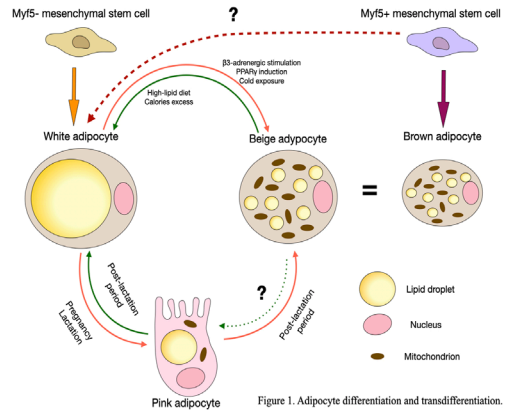

Fig. 1. *Adipocyte differentiation and transdifferentiation.*

Adipocyte origin

BAT develops during embryogenesis from the mesoderm, while BeAC are formed from WAC in the postnatal period. White preadipocytes develop from Myf5-negative progenitor cells during embryogenesis; differentiation of white preadipocytes in mature WAC occurs mostly postnatally.[28]

The differentiation of WAC into BeAC is called WAT remodeling or browning.[13] BrAC and BeAC have different origins, but similar morphology and metabolic characteristics. Classic BrAC are derived from the Myf5-positive muscle progenitor cell line.[20] BrAC are usually localized together with WAC, for example, in the interscapular and perirenal regions. Moreover, in response to prolonged cold exposure or stimulation of β-adrenergic receptors, WAC acquire UCP-1 expression, which results in a browning phenomenon and occurrence of Myf5-negative brown cell type.[29] It should be mentioned that cold stimulation or administration of β3-adrenergic receptor agonists unevenly increases the level of UCP-1 expression in white cells. This phenomenon is called the "Harlequin effect"—AT looks heterogeneously stained after immunostaining assay for UCP-1.[13,30] However, chronic exposure to cold weakens this effect (cells are stained more evenly), which may be associated with increased expression of heat shock proteins protecting adipocytes from damage caused by overheating.[31] At the same time, browning of neighboring WAC is efficiently stimulated, which allows heat to be distributed between cells more equally.[32]

According to Jeffery et al.,[33] the pathway of preadipocyte differentiation may depend on niche environmental local factors. It has been shown that donor preadipocytes isolated from subcutaneous and visceral depots undergo subsequent differentiation according to the injection site under high fat diet conditions, without any difference in place of origin; preadipocytes keep the ability to differentiate only in visceral, but not in subcutaneous, fat depot.[33] According to recent studies, An increase in PPARγ expression level stimulates adipogenesis in fibroblast cultures. Nevertheless, it is unable to initiate adipogenesis in vivo in cases of the limited differentiation ability (in subcutaneous fat depot or during low fat diet).[34] Based on this data, it is possible to raise the questions: Which factors guide differentiation of the precursor cells, what are the sources of these factors, and how is adipocyte differentiation regulated in different fat stores? These questions remain unresolved. Furthermore, different populations of stromal cells in various fat depots consist of several subpopulations, including cells with anti-adipogenic properties, which was shown in single-cell transcriptome profiling analysis.[35,36] Thereby, AT is characterized by high plasticity and heterogeneity, which depends on different endogenous and exogenous factors. This phenomenon can be confirmed by differential gene expression in various human fat depots and different factors, affecting adipogenesis, as well as genes, differentially expressed in adipocyte progenitor cells.[37,38]

Stages of adipogenesis

Adipogenesis is a process of multipotent MSC differentiation in adipocytes.[36] WAC arise from MSCs localized in AT stroma. When MSCs become preadipocytes, they lose the ability to differentiate in other mesenchymal clones. This stage of adipogenesis is the determination phase. The second stage is terminal differentiation; in this step, preadipocytes acquire the characteristics of mature adipocytes by accumulating lipid droplets and gaining the ability to respond to endocrine stimuli.[39] Thus, MSCs differentiate into lipoblasts, then into preadipocytes and ultimately into mature adipocytes.[1] According to the modern theory of the origin of adipocytes, precursor cells, endotheliocytes and pericytes are localized in the walls of adipose capillary beds.[40] Capillary explants from human AT, as well as a suspension of individual microvessel cells, may form adipocyte precursors, able to autonomously differentiate into mature fat cells.[41] Moreover, according to experimental data, human adipocytes may de-differentiate into endothelial cells;[42] endothelial cells can be de-differentiated into mesenchymal cells, which give rise to adipocytes, chondrocytes and osteoblasts.[43] The mesenchymal-endothelial transition is associated with activation of the BMP/TGFβ signaling pathway.[44] Besides, according to the latest clinical and experimental research, at least several subpopulations of adipocytes are derived from hematopoietic cells of BM.([45] In addition, some studies have revealed a mesothelial origin of visceral adipocytes.[46] Nevertheless, the problem of adipocyte biogenesis is still unsolved and being intensively investigated.

Adipogenesis of white and brown adipocytes requires activation of the same key transcription factors: PPARγ, C/EBPs, Krüppel-like factor, as well as the signal transducers and activators of transcription (STAT) proteins.[1] Differentiation of adipocytes is also regulated by BMPs, which belong to the TGFβ superfamily. In this case, the differentiation of WAC is regulated by BMP-2 and BMP-4, while BMP-7 is a major factor in the differentiation of

preadipocytes into mature BrAC. Moreover, an important regulator of brown and white adipocytes maturing is the activated p38 signaling pathway.[28]

Some of the universal markers of preadipocytes in WAT are PDGFRα and PPARγ, expressed in progenitor cells, but not in mature adipocytes. PDGFRα+ and PPARγ+ cells are localized in the capillary walls in WAT depots. These cells are called pericytes.[34] PPARγ+ cells resemble smooth muscle cells of the blood vessels and express the same markers, such as αSMA (α-smooth muscle actin) and PDGFRβ.[47] Modern studies have shown that turning αSMA+ and PDGFRβ+ cells into WAC may be induced in vivo by a high fat diet.[48] However, a recent analysis of single-cell transcriptome profiling showed heterogeneity of adipocyte precursors from different fat depots. Additionally, there were described different stimuli, which are able to activate the differentiation of adipocyte precursors into mature adipocytes.[49]

Early studies on fibroblast cultures showed that BMP2 and BMP4, by activating transcription factor SMAD4, are able to initiate the differentiation of fibroblasts into adipocytes in vitro.[50] SMAD4 stimulates the transcription of PPARγ, the main regulator of adipogenesis. On the other hand, adipogenic fibroblasts are characterized by increased expression of transcription factor ZFP423 (zinc-finger transcription protein 423), which enhances the sensitivity of fibroblasts to pro-adipogenic BMP signaling.[51] Furthermore, expression of ZFP423 is required for the formation of subcutaneous fat in the fetus, but it is not required for the formation of visceral fat in cases of a high fat diet.[49,52] Many other factors directing fibroblast differentiation into adipocytes have been identified; however, the ways in which their expression is activated and regulated remain mostly unknown.

PPARγ is considered as the key regulator of adipogenesis, since its expression is absolutely required for terminal differentiation of preadipocytes, both in cell cultures and in vivo.[53,54] According to recent publications, there are many molecules that could be considered endogenous PPARγ activator ligands. The most common are polyunsaturated FAs, eicosanoids, and prostaglandins. However, the affinity or concentration of such potential activators in AT is low; therefore, the true physiological ligand PPARγ is still unknown.[55]

One of the most important effects of PPARγ is the activation of transcription factor C/EBPα.[56] According to Freytag et al.,[57] the induction of C/EBPα and PPARγ expression is sufficient for fibroblast differentiation into mature adipocytes in cell cultures.

More than 90% of binding sites PPARγ also bind to DNA and C/EBPα. In order to completely activate transcription of genes expressed in mature adipocytes (for example, genes encoding insulin receptors, glucose transporters, adiponectin, adipocyte fatty acid-binding protein (aP2), perilipins, leptin, and others), PPARγ activates transcription of C/EBPs.[58] However, C/EBPα expression is not required for adipogenesis activation during embryogenesis, possibly due to a high C/EBPβ expression level. The expression of C/EBPα[59] is absolutely essential for all forms of adipogenesis in adult organisms also (Fig.2)

Adipocytes are energy storing mesenchymal cells. Adipogenesis may be divided into two stages: determination and terminal differentiation. In the first step, MSCs become able to differentiate into fat storing cells. Terminal differentiation is characterized by special morphological and functional properties of adipocyte development, including accumulation of lipid droplets.

WAT is considered as the most dynamic tissue in the human body, possessing high plasticity.[60] It is known that preadipocyte differentiation is not a unidirectional process, and under certain conditions, adipocytes are able to de-differentiate into fibroblast-like cells (Fig.2). This process is observed during wound healing, tumor growth and lactation.[61-63] Additionally, the alveolar cells of the mammary gland may undergo the process of transdifferentiation (Fig.1). Furthermore, alveolar cells have shown an ability to transdifferentiate into BeAC in the early post-lactation period.[30]

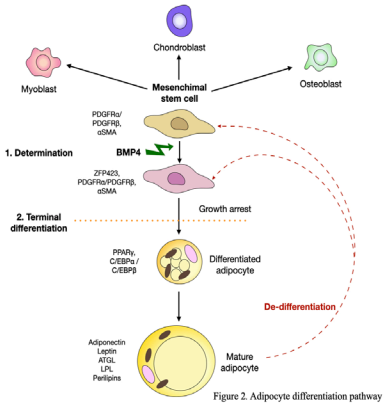

Fig. 2. *Adipocyte differentiation pathways.*

Regulation of adipogenesis

The increase in AT volume could be achieved in two ways: hypertrophy, the adipocytes' size increase; and/or hyperplasia, the cell number increase. According to the literature data, the number of adipocytes is genetically determined, thus, the number of WAC remains constant in adulthood both in lean and obese people and even after noticeable weight loss. Therefore, the fat mass in adults could be increased mostly by accumulation of lipids in already developed fat cells.

Recent studies in rodents based on radiolabeling showed subcutaneous preadipocyte differentiation during the embryonic period. The main mechanism of postnatal fat mass increase was suggested to be associated with hypertrophy.[65] At the same time, the development of visceral AT occurs mainly postnatally, equally both by hyperplasia and hypertrophy.[66] Moreover, about 10% of adipocytes are annually renewed, and this process does not depend on BMI, age or environment.[64]

The early recruitment of new fat cells depends on cross-talking between Wnt and BMP-4 signals. Wnt amplifies proliferation of preadipocytes, while BMP-4 is responsible for their terminal differentiation into mature adipocytes. Wnt proteins inhibit adipogenesis, suppressing the expression of CEBP-α and PPAR-γ.[60] Wnt proteins belong to a family of

secreted glycoproteins regulating tissue remodeling in adults. Besides, Wnt signaling is required for cross-communication of fat cells. Wnt is the chief regulator of β-catenin, which participates in the coordination of gene transcription. In case of the Wnt low level, cytoplasmic β-catenin is phosphorylated by casein kinase I and kinase of glycogen synthase 3-β, which leads to its ubiquitination and subsequent proteasome degradation.

Wnt binds to Frizzled (FZD) receptors and LDLR5/6, which inactivates the degradation complex. This leads to β-catenin hypophosphorylation and its translocation into the nucleus, where it binds to the lymphoid enhancer binding factor 1/T cell-specific factor (LEF1/TCF) to activate Wnt target genes (Fig.3).

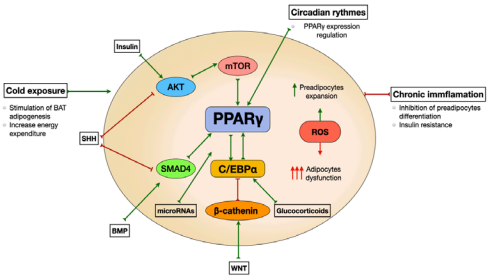

Figure 3. Regulation of adipogenesis

Fig. 3. *Regulation of adipogenesis.*

Intracellular pathways and extracellular stimuli affecting adipogenesis. Upregulation stimuli are indicated by green indicators. Downregulation stimuli are indicated by red indicators.

This damage to the signaling pathways is associated with adipogenesis dysregulation and the organism's inability to regulate the accumulation of lipids in subcutaneous AT in response to environmental factors. This leads to hypertrophy, dysfunction and the development of insulin resistance. GLUT-4 synthesis and activation decrease and glucose transport into adipocytes is reduced.[67]

One of the most crucial roles in adipogenesis regulation belongs to ROS, the formation of which affects stability of the HIF-1α that inhibits PPAR-γ.[60] It has also been shown that ROS in physiological concentrations has the ability to enhance insulin signaling, which stimulates adipogenesis,[68] However, high ROS levels, produced by NADPH oxidases during obesity, are associated with development of insulin resistance and suppression of adipogenesis.[69]

MicroRNAs provide an additional mechanism of adipogenesis control.[60] According to recent studies, some miRNAs inhibit the expression of leukemia inhibitory factor (LIF), which leads to adipogenesis activation. For example, microRNA-130, microRNA-378 and microRNA-27 control the expression of adipogenic and lipogenic genes.[60]

The most important pro-adipogenic properties reside in glucocorticoid hormones and insulin, which directly affect the expression of PPARγ and C/EBPα transcription factors, guiding preadipocyte differentiation.[70,71] In contrast, the Hedgehog signaling pathway inhibits adipogenesis by suppressing pro-adipogenic signaling cascades.[72] Other regulatory factors of adipogenesis include cold-dependent β3-adrenergic stimulation,[73] proinflammatory molecules, TGFβ, and NO. The physiological concentrations of these factors are important stimulators of adipogenesis; however, chronic inflammation suppresses adipogenesis as a high level of proinflammatory factors accelerates cell aging and enhances metabolic imbalance.[74,75] PPARγ expression depends on circadian rhythms; therefore, development of obesity and dysregulation of adipogenesis are associated with disruption of circadian rhythms [76] (Fig.3).

Adipocyte senescence and death

Human adipocytes live for approximately 10 years. During this period triacylglycerols (TAGs) in adipocytes are renewed about 6 times. It has been shown that adipocytic TAGs are constantly metabolized and there are no constitutive TAGs that do not undergo lipolysis.[77]

Age-related BAT reduction occurs due to a reduction in stem cells' reproductive capacity, diminished production of triiodothyronine and low conversion of thyroxine to triiodothyronine, mitochondrial dysfunction and decreased sympathetic activity of the nervous system.[78] The modern developmental theory states that chronic inflammation should be considered as a key factor of aging or tissue senescence.[79] The cell number of macrophages and T-lymphocytes, secreting certain factors, increases in AT, and that leads to damage and degeneration of the surrounding cells and development of chronic inflammation (called "inflammaging").[80]

Senescence of adipocytes has been studied based on profiling of gene and secretory β-galactosidase activity in paired biopsies of subcutaneous and omental AT in severely obese patients. The activity of β-galactosidase is seven times higher in the subcutaneous AT than in the omental AT. Furthermore, it has been shown that higher β-galactosidase activity is associated with a high serum leptin level, severe dyslipidemia, and development of insulin resistance. According to Rouault et al.,[81] levels of several factors, such as IGFBP-3, PAI-1, CCL2, and IL-6, were increased in senescent subcutaneous AT. Senolytic treatment reduced β-galactosidase activity and led to normalizing the levels of the factors.[81]

Obese people have a variety of metabolic changes. As adipocytes increase, cells undergo mechanical stress, since they begin to contact neighboring cells and components of the extracellular matrix.[82] In addition, hypoxia develops due to limited diffusion of oxygen to adipocytes. Mechanical and hypoxic stress leads to the development of inflammation, which is accompanied by increased lipolysis, secretion of inflammatory cytokines and decreased secretion of anti-inflammatory adipokines such as leptin and adiponectin.[82] In addition, the size of adipocytes is positively correlated with the number of CD206+ macrophages involved in phagocytosis of dead cells.[83] Thus, not the obesity itself, but the exact size of adipocytes is a key factor responsible for their death. Death size is the cell size; when that size is reached, cell death is induced. Visceral adipocytes have a smaller critical death size than subcutaneous adipocytes.[30]

In hypertrophied adipocytes, a number of important pathological changes are observed: changes in the number and size of mitochondria, hypertrophy of the endoplasmic reticulum and the Golgi apparatus, which is associated with other causes of overproduction of proinflammatory factors, such as MCP-1, MIP-1α, MCP-2, MCP-3, RANTES. Accumulation of cholesterol granules and overproduction of collagen and amyloid in the extracellular matrix also occurs.[84-86] Cholesterol presence and modification of cell organelles are potential molecular patterns associated with cell damage (DAMPs, damage-associated-molecular patterns) because they act as activators of NLR receptors (Nucleotide oligomerization domain [NOD]-like receptors). These receptors together with procaspase-1 and PYCARD (apoptosis-associated speck-like protein containing a CARD [C-terminal caspase recruitment domain]) form a unified protein complex.[87] Activation of NLRs leads to the formation of active caspase-1, which initiates a special type of cell death associated with hyperinflammation—pyroptosis.[88] Given that the number of cells producing inflammatory factors increases with age, more adipocytes undergo pyroptosis, which leads to greater hypertrophy of remaining cells and the development of metabolic disorders associated with their hypertrophy.

However, about 20%-30% of the total cohort of obese patients are metabolically healthy people. Moreover, clinical studies have shown beneficial effects of adipogenesis stimulation, improving metabolic health in people with low BMI. For example, people who are predisposed to hypertrophic obesity have a reduced insulin sensitivity; therefore, these patients develop insulin resistance even with normal body weight, without obesity. Besides, inhibition of adipogenesis during adulthood by deletion of PPARγ leads to pathological hypertrophic expansion of WAT in conditions of overnutrition. In contrast, the stimulation of visceral adipogenesis by PPARγ overexpression leads to beneficial effects: decrease in the level of local inflammation and maintenance of normal secretion of adiponectin without weight gain. Additionally, impaired adipogenesis may lead to inability to sequester lipotoxic FAs.[89]

Adipocytes signaling molecules

The endocrine function of AT is well known. AT secretes hormones, cytokines, and protein growth factors like leptin, adiponectin, resistin, visfatin, apelin, asprosin, and FGF21 (Fig.4), all of which affect various organs.. The signaling molecules and target organs are presented in Figure 2. The font is increased for the main signaling molecules. These factors have many targets of action (liver, skeletal muscles, nervous tissue, heart, intestines, pancreas, etc.) and play an important role in their functions and metabolism regulation.[90]

However, new signaling molecules formed by AT were recently identified. These molecules include FAHFAs and diHOMEs.[2] Most studied FAHFAs are compounds of palmitic acid with hydroxystearate. The FAHFA level is increased in mice with high GLUT-4 expression in AT and in animals with a metabolically healthy phenotype. FAHFAs also exhibit anti-inflammatory properties, reducing lipopolysaccharide-induced macrophage activation.[2,91] Lipokine diHOME is a product of linoleic acid metabolism (12,13-dihydroxy-9Z-octadenoic acid or 12,13-diHOME). A high level of 12,13-diHOME is found in WAT, where it increases the utilization of FFA. Physical activity and cold exposure are positively correlated with high levels of 12,13-diHOME in blood serum, due to its regulatory role in the process of free FA oxidation in myocytes.[9,92]

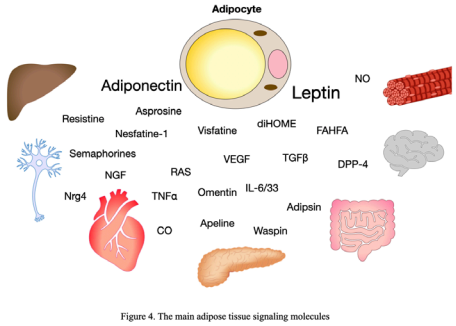

Figure 4. The main adipose tissue signaling molecules

Fig. 4. *The main adipose tissue signaling molecules.*

Conclusion

Despite the extreme complexity of adipocyte origin issues, the molecular mechanisms of their formation, and their metabolic role in AT, the concept of adipogenesis takes shape as a multi-stage process of MSC differentiation. At the same time, preadipocytes are believed to have heterogeneity, plasticity, and perivascular origin. Precursors of adipocytes have also been found outside the main fat depots and play an important role in the processes of regenerating skin, bone, and muscle tissues and regulating their metabolism. An important adipocyte characteristic is an ability to return to the original fibroblast-like cell state, as well as the ability to transdifferentiate into a different type of adipose or non-AT. Adipogenesis and the pathway of mature adipocyte transformation depend on many factors that have systemic (action of hormones, cold stimulation, circadian rhythms) and local nature (influence of the microenvironment, para- and autocrine factors). Rate of adipogenesis and metabolic function of AT have an exceptional impact on the body's health and life expectancy. Adipogenesis plays an important role in general metabolism regulation and allows sequestering lipids, preventing their lipotoxic effect on other tissues. Thus, new therapeutic regulators of adipogenesis development may be potentially useful for the treatment of obesity, type 2 diabetes and other metabolic disorders, as well as for regenerative therapy.

Competing Interests

The authors declare that they have no competing interests.

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REVIEW ARTICLE

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Men's Reproductive Health: Oxidative Stress and the Effectiveness of Antioxidant Therapy

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Abstract

This review article surveys and summarizes the literature data concerning the problems of deterioration of the main indicators of men's reproductive health and the effectiveness of therapy with drugs with antioxidant properties.

The analysis of domestic and foreign literature confirms that oxidative stress accompanies and/or is one of the pathogenetic links in the development of many types of reproductive pathology in men. The health status of the male population is influenced by the following factors: lifestyle, existing diseases, the state of the environment, and the genotype of the population. The interaction of several weak, but unidirectional, factors creates a high risk of developing male reproductive pathology. The polyetiological nature of fertility disorders in men causes certain difficulties in creating adequate methods of treating this pathology and causes multicomponent biocomplexes to appear on the pharmaceutical market that contain active substances that can affect the quality of the ejaculate.(**International Journal of Biomedicine. 2021;11(3):333-336.**)

Key Words: men • reproductive health • oxidative stress • infertility • antioxidant therapy

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Today, there is an important trend in the demographic policy of the State to preserve the reproductive health of the population. In recent years, there has been a decline in the absolute population because its reproduction has been reduced, and each subsequent generation is smaller than the previous one.[1-5]

Over the past few years, a progressive decline in fertility among adult men has been noted both in Russia and abroad, and therefore the standards of the spermogram have been revised in the direction of reducing the quantitative and qualitative indicators of the ejaculate.

It is known that different indicators of the ejaculate, such as the number, motility, and morphology of sperm, are sensitive to the action of free radicals. Spermatozoa were the first type of cells in which the formation of free radicals was described.[2,5,6]

Reactive oxygen species (ROS) normally form the spermatozoa themselves, and ROS play an important physiological role in the mechanism of the acrosomal reaction, that is, they are necessary in the process of the sperm penetrating the egg. Along with the formation of ROS, they are continuously deactivated by a special system of antioxidants contained in the seminal plasma, which provides a balance between the oxidant and antioxidant systems in the vas deferens. Violation of this balance inevitably leads to a deterioration in fertility and as a result to a violation of the reproductive potential.[4,7-9]

Spermatozoa are more sensitive to oxidative stress than any other cells, due to the small volume of the cytoplasm, low concentration of antioxidants, and a large amount of polyunsaturated fatty acids, which are easily subjected to peroxidation.[6-8] In addition, the structural features of spermatozoa are such that antioxidant enzymes are not able to protect the cell membrane at the levels of the tail and

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acrosome.[4,8,9] In response to the widespread hypothesis that free radicals play a leading role among most processes of the body, it was assumed that the intake of antioxidants can reduce or prevent the development of oxidative stress and slow down the process of cell destruction. This will stop the progression of many diseases and prolong the active years of a person's life, and in the case of infertility – improve the characteristics of spermatozoa and the quality of the ejaculate as a whole.[10,11] It has been shown that there are vitamins, minerals, amino acids, and other organic compounds that can reduce oxidative stress by enhancing metabolic processes, activating nuclear-cytoplasmic transport, and reducing the activity of inflammation and weakening autoimmune reactions; and that taking drugs containing these compounds in infertile men leads to an improvement in sperm parameters, restoration of the sperm function and an increase in the frequency of pregnancy.[12]

We analyzed the possibility of using antioxidant supplements to prevent and treat infertility, using publications included in the Cochrane Library, EMBASE, MEDLINE, PubMed, and eLibrary databases. The most effective and important classes of dietary supplements are natural dietary antioxidants and dietary supplements in preparations because the formation of free radicals under oxidative stress plays a confirmed role in the violation of sperm function.

The groups of antioxidants are classified as endogenous and exogenous. Endogenous are divided into enzymatic, such as catalase, superoxide dismutase, glutathione peroxidase, and non-enzymatic, such as glutathione, vitamin E, vitamin A, vitamin C, coenzyme Q10, and L-carnitine. Exogenous antioxidants (vitamins E and C, carotenoids) enter the body with food.[5,11] Oral administration of antioxidants significantly reduces the index of DNA fragmentation, including under conditions of oxidative stress.[13]

A decrease in the concentration of malondialdehyde in semen, a product formed under the action of ROS during the degradation of polyunsaturated fats and a marker of oxidative stress, was noted by S.A. Suleiman et al. (1996), while establishing a concomitant increase in sperm motility in men with asthenozoospermia and a higher frequency of natural conception, compared to the control group. Multicomponent biologically active complexes containing L-carnitine, vitamin E, folic acid, and zinc in increased dosages have been widely used to correct oxidative stress in infertile men. According to the results of the use of the complex, patients showed an increase in the volume of ejaculate by 10% and in the concentration of spermatozoa by 15.6%, and a decrease in the dilution time by 32%, against the background of a significant decrease in the levels of ROS and fragmentation of the DNA of spermatozoa.[14]

Micronutrients can help reduce inflammation, weaken autoimmune processes, improve intermediate metabolism, activate nuclear-cytoplasmic transport, and restore the integrity of sperm membranes. The most significant work is the Cochrane review, which analyzed the cases of 2,867 couples who participated in 34 studies. There was a statistically significant increase in the frequency of pregnancy and childbirth in subfertile couples. Micronutrient supplements may also be useful for patients who resort to assisted reproductive technologies.[13-15]

The review studied and evaluated the effectiveness of the basic sperm supplement containing glutathione, L-carnitine, L-arginine, coenzyme Q10, α-tocopherol, folic acid, zinc and selenium in patients planning to resort to IVF. The tests were taken initially and after 12 months, before the use of assisted reproductive technologies. Semen samples were obtained from 147 patients. The results of the analysis showed a sharp increase in the indicators of motility and the total number of spermatozoa in patients with oligoastenoteratozoospermia.[7,13,16]

A study by Neymark et al.[17] showed the effectiveness of a dietary supplement composed of a complex of trace elements and vitamins: selenium, zinc, vitamin E, vitamin C, and beta-carotene. Zinc has a high bioavailability due to the organic form of lactate, and the synergism of the components enhances the antioxidant effect of the drug as a whole. Three months after treatment, the following indicators of ejaculate were obtained in patients: an increase in the volume of ejaculate by 59%, an increase in the number of spermatozoa by 79%, an increase in the number of viable spermatozoa by 28%.[17] The resulting positive effect is probably due to the antioxidant effect of zinc by the activation of glutathione peroxidase of spermatozoa, since sufficient activity of this enzyme ensures normal maturation and motility of spermatozoa.[18] The replacement of zinc deficiency also leads to an increase in the overall activity of the antioxidant system and an increase in the number of pro-inflammatory cytokines.[18,19]

Quite a lot of biologically active additives (dietary supplements) have been developed that affect certain parts of the male reproductive system or certain diseases.[17-19] Taking into account the pathogenesis of disorders that occur in the pathology of the male reproductive system, as well as the peculiarities of the effect of biologically active substances on the body, a multicomponent natural complex containing plant extracts, B vitamins (B5, B6, B12), vitamin C, and L-arginine was created.[20] The presented complex is recommended to be used to stimulate the synthesis of testosterone in case of its insufficiency in various etiologies. Unlike synthetic hormonal drugs, it does not violate the physiological mechanisms of hormonal regulation, increasing the level of androgens only when they are deficient in the body. Oral intake of a dietary supplement can play a direct role in improving the parameters of sperm and restoring its function. Studies of targeted nutraceuticals, which include fat- and water-soluble antioxidants, amino acids, and metabolic cofactors, have shown a 10.2% improvement in the DNA fragmentation index. There was also a 70% increase in sperm count and an 85% increase in motility. The volume of ejaculate increased from 2.6 ml to 4.3 ml, an increase of 39.5%.[20]

A new biocomplex, including retinol, α-tocopherol, glycyrrhizic acid, coenzyme Q10, arginine, carnitine, carnosine, zinc, and selenium, has recently appeared on the pharmacological market; this complex improves the quality of the ejaculate by affecting various parts of the pathogenesis of fertility disorders.[21] The synergistic effect of the components of the biologically active complex is due to the use of Actielease nanotechnology in the production (separation

of active ingredients into microscopic nanoparticles), which provides an optimal concentration of components, high bioavailability and stability of the composition. The components of the drug contribute to the improvement of microcirculation, indirectly stimulate the release of testosterone, have a detoxifying, antihypoxic, reparative effect, normalize lipid and carbohydrate metabolism, have a powerful antioxidant effect, are able to inhibit apoptosis, and protect the cells of the reproductive system.[17,22] After a course of therapy (30 days), patients showed a significant increase in all indicators, compared to the initial parameters (the volume of ejaculate increased by 78.2%, the number of spermatozoa in 1ml increased by 80.7%, the viability of spermatozoa by 1.35 times, the number of spermatozoa with normal morphology – by 1.57 times).[17] There was a statistically significant decrease in sperm DNA damage (in absolute values-by 4%), while the positive dynamics of DNA fragmentation during treatment occurred in 67% of men. Also, during treatment, the severity of oxidative stress significantly decreased, which was confirmed by a decrease in ROS production by washed spermatozoa, on average by 2-5 times, compared to the baseline level.[21]

Modern multicomponent complexes for improving spermatogenesis and increasing male fertility include a combined drug containing (in addition to vitamin E, coenzyme Q10, carnitine, selenium, and zinc), docosahexaenoic acid, which not only has antioxidant properties, improving the composition of membranes, but also affects various parts of spermatogenesis disorders, preventing DNA fragmentation and increasing sperm motility and survival.[23] The criterion for the effectiveness of therapy is the improvement of the main indicators of the spermogram: the volume of ejaculate, the concentration of spermatozoa, the proportion of spermatozoa with translational movement, and the normal morphology of spermatozoa.

According to the research data of Korenkov et al.,[23] no cases of drug intolerance were recorded. The spouse of one of the patients in the main group became pregnant 11 weeks after the start of therapy. However, in the study of Bozhedomov et al.,[24] on the contrary, there was no significant effect of this drug on spermogram parameters, sperm DNA fragmentation, or the percentage of pregnancies that occurred.

Despite the rather diverse composition of the presented supplements, none of them contain such an important substance for male fertility as vitamin D.[25] Vitamin D deficiency is observed in people of all ages, including men of reproductive age, in many countries of the world, including Russia. There is evidence that vitamin D deficiency negatively affects sperm quality (reduced overall and progressive sperm motility, total motile sperm count) and hormone function, and the frequency of pregnancy and childbirth is significantly higher in couples with normal vitamin D levels.[25] Enzymes that metabolize vitamin D are simultaneously expressed in Sertoli cells, Leydig cells, sperm cells, and epithelial cells lining the male reproductive tract. The somatic or germ cells of the testis appear to be able to synthesize and activate vitamin D locally.

By means of genomic mechanisms, 1,25(OH)2D3 regulates the expression of androgen biosynthesis enzymes in Sertoli cells; by means of a non-genomic mechanism (in particular, activation of the signaling cascades of protein kinases A and C, MEK kinase), 1,25(OH)2D3 stimulates the absorption of calcium and increases the activity of gamma-glutamyl transpeptidase. In the studies of Wadhwa et al.,[26] it was noted that vitamin D increases sperm motility because it directly affects sperm cells, including non-genomic-controlled modulation of intracellular calcium homeostasis and activation of molecular pathways involved in sperm motility, condensation, and acrosome reaction. Even at low doses (200 IU/day, 3 months), vitamin D in combination with calcium (600 mg/day) significantly improved the condition of patients with idiopathic oligoastenozoospermia, improving, in particular, sperm motility.

Spermatogenesis is an energy-intensive process that requires a sufficient and balanced amount of vitamins and minerals in the body. The results of the analysis of domestic and foreign authors show that a positive trend in fertility disorders in men is given by phytotherapy, treatment with micronutrients, and therapy with antioxidant drugs containing retinol, α-tocopherol, vitamin D, glutathione, coenzyme Q10, L-arginine, L-carnitine, carnosine, selenium, zinc, B vitamins, docosahexaenoic acid, glycyrrhizic acid, and folic acid in various combinations.[27,28]

However, there is not enough data to provide convincing recommendations based on the high reliability of the evidence regarding the dosage, frequency, and duration of each drug. Do not forget that antioxidants, despite their versatility, are not a panacea, but a very subtle regulatory tool. Studying the regulatory system as a whole, it is necessary to determine which cases can be treated with antioxidants alone and which require complex therapy, in which other biologically active substances are used in addition to antioxidants.[27,29]

The presented analysis of publications devoted to the conservative treatment of male infertility and fertility disorders demonstrated that most of the drugs are effective, but at the same time, more study is required to improve pharmacological achievements in the fields of endocrinology and urology. This requirement determines the direction of further research.

Competing Interests

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Pediatrics

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Problems in Diagnosing Autism Spectrum Disorders in the Irkutsk Region

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Abstract

The purpose of this study was to evaluate the effectiveness of methods for studying the neuropsychiatric development of children in regards to screening for the diagnosis of autism spectrum disorders (ASD) with the example of educational institutions in the Irkutsk region.

Methods and Results: Two groups of children in educational institutions of the Irkutsk region regional center were studied: 187 children of preschool age (from 5 to 6 years) and 154 children studying at school (from 7 to 11 years). This study used the methods of neuropsychiatric research used by the pediatric service and the methods of screening diagnosis of autism spectrum disorders (ASD). It was shown that the level of obvious anxiety of schoolchildren (according to the scale developed by A.M. Prihozhan) was higher than in preschoolers and was 10.3±0.1% in girls and 10.6±0.2% in boys. Bad appetite was observed in 50.3±3.7% of preschool children and in 56.5±4.0% of schoolchildren. The selective appetite was observed in 16.6±2.7% of preschool children and in 7.1±2.1% of schoolchildren. The study of hyperactive traits showed that only 5.3±1.6% of parents of preschool children noted hyperactivity in their children while educators considered that 22.5±3.1% were hyperactive. The parents noted hyperactivity in 9.7±2.4% of children, and the teachers noted hyperactivity in 21.7±3.4% of children. The intellectual development of children, according to the Raven test, showed that the average IQ was observed in 47.6±3.7% preschoolers, and below-average IQ in 52.9±3.7% preschoolers. At the same time in schoolchildren, below-average IQ was found in 48.7±4.0% and average IQ in 51.3±4.0%.

Conclusion: The study of the development of children's mental processes and behavior is not informative for the early detection of ASD. Likewise, the screening methods for the detection of ASD known today and available to the pediatric service and based on the detection of social and communication disorders are poorly informative. However, if we consider ASD as a manifestation of genetic and cytogenetic pathology, we should look for screening methods in the field of genetics.(**International Journal of Biomedicine. 2021;11(3):337-341.**)

Key Words: autism spectrum disorders • neuropsychiatric development • screening methods

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Introduction

The diagnosis of autism spectrum disorders (ASD) has been discussed more and more frequently in scientific and public circles as of late.(1) Unfortunately, despite the almost century-old history of autism, our knowledge of it does not allow us to provide high-quality medical and pedagogical assistance, nor does it allow us to diagnose ASD in time. Attention should also be paid to the problem of conflicting opinions among different categories of specialists regarding the prevalence of ASD in children. Because of this, psychiatrists rarely rush to make a diagnosis until the clinical picture becomes obvious for its formulation, missing the time span for treatment and rehabilitation. At the same time, the parent community is divided into two camps: those who still hope that the signs of neuropsychiatric development disorders in the child will disappear over time, and are in no hurry to seek psychiatric help, and those whose children were diagnosed after many years of observation.(2)

According to the data of European researchers, between 5 to 11.8 children with ASD per 10,000 children

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are registered.[3] In the United States, there are registered up to 60 per 10,000 children.[3,4] With this in mind, the opinion of Russian researchers on the prevalence of ASD is very different.[5]

Due to the regulatory documents adopted in Russia, pediatric care almost completely skips diagnosis of ASD. The clinical guidelines for ASD published in 2020 recommend that diagnostics be carried out almost exclusively on the basis of behavior analysis and psychological tests.[6]

An early diagnosis is necessary in the first 18-24 months of life for the successful correction and treatment of ASD.[7] However, the diagnosis of ASD in Russia occurs much later, almost when the full clinical picture of the disease unfolds—when the child has already entered school and difficulties in adapting to school education are revealed, or even as late as when puberty starts.

As has been noted above, at present, psychiatrists in Russia do not use actual screening methods for ASD when examining a child for the first time at 2 years of age. In our study, we attempted to analyze the state of diagnosis of ASD in the Irkutsk region and to determine the suitability of the selected methods for diagnosing the neuropsychiatric development of children for screening studies of ASD.

The purpose of this study was to evaluate the effectiveness of methods for studying the neuropsychiatric development of children in regards to screening for the diagnosis of ASD with the example of educational institutions in the Irkutsk region.

Methods

Two groups of children in educational institutions of the Irkutsk region regional center were studied: 187 children of preschool age (from 5 to 6 years) and 154 children studying at school (from 7 to 11 years). The new clinical guidelines for the diagnosis and treatment of ASD published in 2020 [6] recognize three groups of disorders as key manifestations of ASD: disorders in the field of social interaction; communication (anomalies in communication); and limited, stereotypical, repetitive behavior.

In addition to these specific diagnostic features, children with ASD often have a number of non-specific problems, such as phobias, sleep and eating disorders, aggression, and autoaggression, which should be defined as comorbid conditions for ASD. Therefore, the study examined and evaluated the indicators of children's behavior, the characteristics of their personality and behavior, the presence of aggressiveness and hyperactivity (by interviewing parents and teachers using the evaluation scale for hyperactivity),[8,9] characteristics of sleep and nutrition, intellectual development of children (with the help of progressive Raven matrices),[10-13] as well as mental performance.[8]

The neuropsychiatric development of children was studied to identify the morphofunctional abnormalities. The assessment was carried out according to the protocols of the pediatric service. The motor sphere was studied, assessing reflexes and the accuracy of movement coordination; the sensitive sphere, determining pain, temperature, tactile, proprioceptive sensitivity, and stereognosis; the vegetative sphere, assessing skin sweating, temperature, trophism, and dermography, and the orthoclinostatic test.[12,13]

Summarizing the data of the assessment of neuropsychiatric development, groups of children were identified in accordance with the indicators, using the method of distribution of neuropsychiatric development groups adopted in Russia:

Group 1 - Neuropsychiatric development corresponds to or outstrips age

Group 2 - Initial abnormalities in neuropsychiatric development

Group 3 - Pronounced deviations

Group 4 - Severe deviations

The study was conducted by observing children as well as by interviewing children and parents.

Personal anxiety was studied using the scale of explicit anxiety according to A. M. Prihozhan.[11] Screening diagnostics of ASD used the CARS scale (Childhood Autism Rating Scale, translation and adaptation by Morozova and Dovbnya).[14]

The study was conducted in accordance with ethical principles of the WMA Declaration of Helsinki (1964, ed. 2013). Written informed consent was obtained from the participant's parent/guardian.

Statistical analysis was performed using the Statistica 10 software package (Stat-Soft Inc., USA). Analysis of the distribution of values obtained was performed using the Kolmogorov-Smirnov test. The mean (M) and standard error of the mean (SEM) were deduced. For data with normal distribution, inter-group comparisons were performed using Student's t-test. 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's chi-squared test. A probability value of $P<0.05$ was considered statistically significant.

Results

When studying anxiety in preschoolers, it was found that children on the scale of obvious anxiety had 9.6±0.3 points, in boys slightly more – 9.8±0.3 points. The study of anxiety in schoolchildren showed that the level of obvious anxiety is higher in them than in preschoolers and was 10.3±0.1 points in girls and 10.6±0.2 points in boys.

The interpersonal anxiety was 4.6±0.4 points in preschool children and 5.4±0.2 points in schoolchildren, self-assessment - 3.2±0.2 points in preschool children and 4.9±0.4 points in schoolchildren ($P<0.05$). The interpersonal anxiety was higher in schoolgirls than in boys (4.2±0.6 vs. 2.8±0.4 points, respectively).

The use of the method of projective diagnostics of anxiety according to A.M. Prihozhan (using situational images), supplemented by sociometric studies, revealed a direct relationship between anxiety and low self-esteem in schoolchildren (r=0.57).

The observations during wakefulness and daytime sleep showed that 63.6±3.5% of children in kindergarten were constantly active during wakefulness, 20.3±2.9% were passive and 16±2.7% were irritable. Among schoolchildren,

the indicators of the nature of wakefulness were distributed as follows: active (49.4±4.0%), passive (23.4±3.4%), irritability during wakefulness (27.3±3.6%).

The distribution of individual character traits in preschool children was as follows: disinhibition (19.8±2.9%), cruelty (24.1±3.1%), aggressiveness (36.9±3.5%), and shyness (10.7±2.3%). About 35.8±3.5% of children were easily trained, 9.6±2.2% were not trained, 23.5±3.1% of children showed curiosity. Other individual traits were distributed as follows: affectionate (28.9±3.3%), kind (32.6±3.4%), rude (25.7±3.2%), contact (27.8±3.3%), obsessive (21.9±3.0%), and fearful (15.0±2.6%) (Fig. 1).

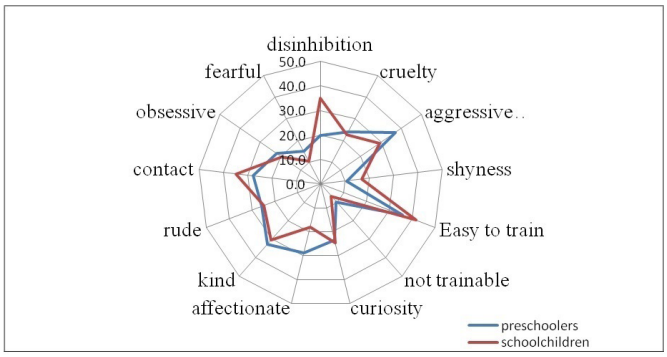

Fig. 1. *The distribution of individual character traits in children*

Among schoolchildren, the individual traits were distributed as follows: lack of inhibition (35.1±3.8%), cruelty (22.7±3.4%), aggressiveness (29.2±3.7%), shyness (16.9±3.0%). Easily trained children consisted of 41.6±4.0%, not trained - 6.5±2.0%, and curiosity was shown by 24.7±3.5% of children. Other individual traits were distributed as follows: affectionate (18.2±3.1%), kind (30.5±3.7%), rude (24.7±3.5%), contact (31.8±3.8%), obsessive (19.5±3.2%), and fearful (10.4±2.5%) (Fig. 1).

In view of the fact that sleep disorders can be observed in ASD, studies were conducted on the nature of sleep and ability to fall asleep in children. Due to the peculiarities of the work of educational institutions, sleep was studied only in preschool. It was shown that about 33.2±3.4% of children fell asleep poorly and 81.3±2.9% had shallow sleep.

According to the available literature, the appetite in children with ASD is often unstable or selective. We also studied the appetite in children to clarify the nature of eating disorders in our research. The following results were obtained in preschool children: good appetite (7.0±1.9%), bad appetite (50.3±3.7%), unstable appetite (9.6±2.2%), increased appetite (15.5±2.6%), and selective appetite (16.6±2.7%) (Fig.2).
In schoolchildren, the appetite traits were distributed as follows: good appetite (7.8±2.2%), bad appetite (56.5±4.0%), unstable appetite (14.9±2.9%), selective appetite (7.1±2.1%). The selectivity of appetite was mainly manifested by the avoidance of meat and dairy products.

The identification of indicators of attention deficit using the hyperactivity rating scale showed that only 5.3±1.6% of parents noted hyperactivity in preschoolers while caregivers considered that 22.5±3.1% children were hyperactive. Among schoolchildren, the parents noted hyperactivity in 9.7±2.4% children, the teachers noted hyperactivity in 21.7±3.4% of children.

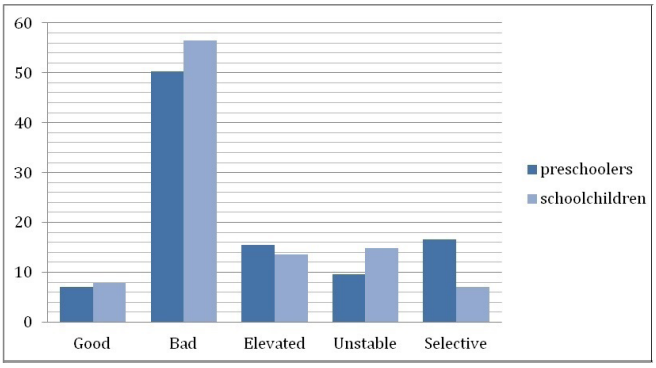

Fig. 2. *The indicators of appetite in children*

The lagging neuropsychiatric development seen in ASD may be one of the first signs of the development of the disease. The obtained data allowed us to divide the children into groups of neuropsychiatric development (Table 1).

Table 1.

Neuropsychiatric development groups of children

Neuropsychiatric development group	Preschool children	Schoolchildren
Group 1	69.5±3.4	55.2±4.0
Group 2	28.3±3.3	40.3±4.0
Group 3	2.1±1.1	3.9±1.6
Group 4	-	0.6±0.6

The study of the levels of intelligence in preschoolers by the Raven test showed that average IQ was found in 47.6±3.7%, below-average IQ in 52.9±3.7% of cases (Figure 3). In schoolchildren, the results were as follows: below-average IQ in 48.7%±4.0 of cases and average IQ in 51.3±4.0% of cases. It should be noted that a low level of intellectual development was not detected, which is a very important result in our study. High levels of intellectual development due to age characteristics were also not identified.

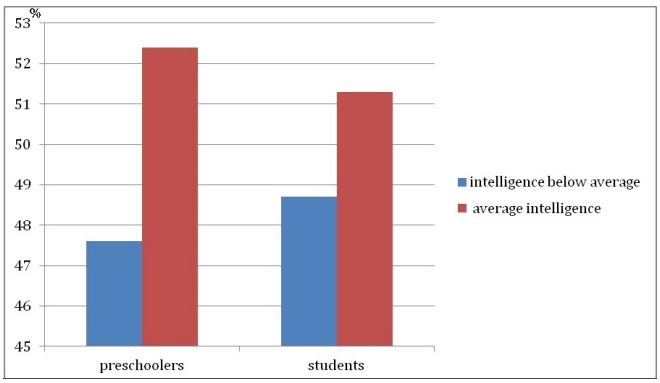

Fig. 3. *The levels of intelligence in children (the Raven method)*

When analyzing the results of the quality of the completed tasks of the Raven test, it was found that children on average perform 38.7±1.2% of the tasks. The indicators of the levels of attentiveness, imagination, and visual difference (discrimination), as well as the establishment of relationships between the figures, was quite high (6.7±1.1 points). Best of all, the children of both groups revealed an analogy between the figures – 7.3±1.1 points. The level of dynamic mindfulness and the establishment of dynamic analogies were slightly lower and amounted to 5.2±0.9 points. In schoolchildren and preschoolers, we found the lowest level of the ability to perceive quantitative and qualitative changes and arrange them according to the regularity of the changes (2.8±0.6 points), the ability to observe the complex quantitative and qualitative development of kinetic, dynamic series, showing abstraction and dynamic synthesis (1.1±0.3 points).

The screening diagnostics of ASD[13] showed that all the examined children scored less than 30 points, i.e. no manifestations of autistic disorders were found. However, 5.3±1.6% of the examined kindergarten children scored between 20 and 25 points, which may indicate that there are individual violations. Deviations from the norm were observed according to such criteria as attitude to people, adaptation to changes, nervousness and fears, verbal and nonverbal communication, level of activity, and consistency of intellectual response.

There were also no obvious signs of autistic disorders among schoolchildren. In the group of schoolchildren, deviations from the norm were more manifested by nervousness and fears, a violation of verbal communication, and the level of activity.

Contrary to expectations, there were more adverse signs in girls than in boys, but the severity of the sign was higher in boys, although the small sample size does not allow us to judge the statistical significance of the differences.

The study of the mental performance of preschool children revealed that the productivity index was 5.4±0.5, errors - 2.4±0.7, and the number of lines viewed - 11.6±0.4. In school children, the productivity index was 11.7±0.6, errors - 1.6±0.6, and lines viewed - 13.2±0.3. The girls looked at slightly fewer lines than the boys, but they also made fewer mistakes.

Discussion

The diagnostic criteria for ASD (defined by ICD-10 and required for diagnosis) are grouped as follows: qualitative disorders of social interaction and changes in communication, limited, repetitive stereotypical patterns in behavior and interests (activities), non-specific problems (fears, phobias, agitation, sleep disorders and eating habits, rage attacks, aggression, and self-harm). One of the diagnostic indicators is the manifestation of these conditions before the child is three years old.[12,13,15]

In our study, in addition to the accepted screening methods, other methods were used that allow us to identify disorders of the neuropsychiatric development of children. However, despite the methods used, it was not possible to identify children with signs of autistic disorders. As noted earlier, foreign and domestic authors claim that, currently, there are between 5 to 11.8 children with ASD per 10,000 children in European countries,[3] and up to 60 per 10,000 children in the United States.[3,4] According to the data provided by the Psychological, Medical and Pedagogical Commission (PMPC) operating in the Irkutsk Region, 66 children with a confirmed diagnosis of ASD are enrolled in educational institutions in the Irkutsk region (as of September 1, 2020), which is 1.14 per 10,000 children.

The epidemiological data on the incidences of autism among adults was not available until recently in Russia. The first results, according to Rosstat, were obtained at the end of 2015: 14,692 children (under the age of 18), which is 0.06% of all children of this age in Russia, and 102 adult patients with ASD, which is less than 0.001% of the entire adult population in Russia; in 2016: 18,224 patients under the age of 18(0.08%) and 96 (<0.001%) adults. In recent years, thanks to pediatric observations and the dissemination of information about the problem of autism, the diagnosis of ASD in children in Russia occurs much earlier than in the past, increasing the number of detected cases of the disease.[16]

Our research has shown that the available assessment tests (as screening tests) and methods for studying the neuropsychiatric development of children are too painstaking and uninformative.

Most children with neuropsychiatric disorders, despite the presence or absence of an established diagnosis, attend specialized or private educational institutions. In educational institutions in general, it is almost impossible to identify children with mild ASD using existing methods. In view of this circumstance, the statistical data differ greatly both in different countries and in the territory of the Irkutsk region in comparison with the data on Russia as a whole.

Another problem in the diagnosis of autism spectrum disorders is the age of children at which it is still possible to make a diagnosis of ASD. For successful treatment, early diagnosis is necessary (preferably earlier than 18 months), and diagnostic criteria, according to the new clinical recommendations,[6] are violations in the field of social interaction; communication (communication abnormalities); limited, stereotypical, repetitive behavior that cannot be formed at the age of less than 18 months. This is a clear contradiction in the formulation of the diagnosis. Our study showed that social and communication skills in preschool and primary school children are not sufficiently formed due to age and may hide pathological violations of these qualities.

Another aspect of the problem of diagnosing autism spectrum disorders is the lack of understanding of the pathogenesis of ASD.[5,17,18] But if we proceed with the postulate that autistic disorders are just a syndrome of impaired social and communication skills, which occurs from certain genetic and epigenetic abnormalities, then the diagnostic criteria for ASD can be genetic disorders. Now, a number of genetic diseases characterized by autistic disorders are already known. These include Fragile X syndrome, also called Martin-Bell syndrome, ,tuberous sclerosis (mutations in either the TSC1 gene or the TSC2 gene), Rett syndrome (mutations in the MECP2 gene),

phenylketonuria (mutations in the PAH gene), Down syndrome (trisomy 21), Prader-Willi syndrome (a deficiency of paternal gene expression on chromosome 15q), Angelman syndrome (a deficiency of maternal gene expression on chromosome 15q), Smith-Magenis syndrome (deletion of chromosome 17p11. 2), DiGeorgi syndrome (22q11.2 deletion syndrome), Phelan-McDermid syndrome (22q13.3 deletion syndrome), Kleefstra syndrome (9q34.3 microdeletion syndrome). Other genetic abnormalities characterized by autistic disorders are likely to be identified. In this aspect, research on somatic health is very important,[19] as well as the study of the pathogenesis of autistic disorders.[19,20]

If we continue to assume that ASD is a manifestation of genetic abnormalities, then screening and diagnosis using currently known methods that lie in the plane of psychiatry and neurology, most likely will not lead to success.

Conclusion

New methods, which identify risk groups for the development of neuropsychiatric disorders at the early stages of a child's development and timely development of optimal algorithms for specialized psychoprophylactic care for each child, could present a promising future in screening diagnostics of ASD in children. This is especially important for the conditions informing education and upbringing when the load on the psyche is increasing many times. In the Irkutsk region, the problem of screening children with ASD remains unresolved—the number of detected cases of ASD most likely does not reflect the actual situation.

In our opinion, it is worth reviewing the known diagnostic approaches to ASD, perhaps changing the routing of patients, transferring diagnostic preferences from the field of psychiatry to the field of genetics. In accordance with this, treatment methods may be found in the field of gene therapy.

The increase in the number of children with ASD cannot but cause concern in scientific and public circles. The search for diagnostic criteria for autistic disorders, despite numerous studies in this area, including our research, remains an urgent area of scientific research.

Competing Interests

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Radiology

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Magnetic Resonance Imaging in Diagnosis of Complications of Renal and Ureteral Injuries in Different Periods of Traumatic Disease

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Abstract

The aim of this study was to evaluate the effectiveness of MRI in diagnosing combined renal and ureteral injuries at different periods of traumatic disease (TD).

Methods and Results: We analyzed the results of diagnostics and treatment of 139 patients (80 women and 59 men) with renal and ureteral injuries aged between 18 and 72 years. There were 67(48.2%) patients in the period of acute reaction to trauma, 40(28.8%) patients with early manifestations, and 32(23%) patients in the period of late manifestations. In 127(91.4%) patients, an urgent plain abdominal X-ray was performed without any preliminary preparation. USI of the abdominal and retroperitoneal space was performed in 108(77.7%) patients in the stage of the primary assessment of renal injury as it was a rapid non-invasive investigation. A whole-body MSCT was performed in 131(94.2%) patients, using the nonionic contrast agents Ultravist (350mg I/ml) and Omnipaque (350mg I/ml). MRI was performed in 125(89.9%) patients, including cases of pregnancy and a medical history of allergies. Contrast-enhanced MSCT had a high diagnostic efficiency in assessing complications in kidney and ureteral injuries at different periods of TD (accuracy of 89.2% for acute reaction, 88.8% for early manifestations, and 89.5% for late manifestations). MRI of the kidneys and ureters was indicated in periods of early and late manifestations of TB to detect renal complications in cases with a discrepancy between clinical manifestations and the results obtained by ultrasound and MSCT (accuracy of 87.5% for early manifestations and 89.9% for late manifestations).(**International Journal of Biomedicine. 2021;11(3):342-345.**)

Key Words: magnetic resonance imaging • multislice computed tomography • trauma • kidney • ureter

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Abbreviations

ADC, apparent diffusion coefficient; **CTU**, computed tomography urography; **MSCT**, multislice computed tomography; **MRI**, magnetic resonance imaging: **MRU**, magnetic resonance urography; **TD**, traumatic disease; **USI**, ultrasound investigation; **UT**, urinary tract.

Introduction

Trauma still remains one of the most frequent causes of mortality in both Western countries and the Russian Federation. According to various authors, retroperitoneal hemorrhage is detected in one-quarter of patients with closed abdominal trauma.[1] In the general structure of traumatism, the cases of combined renal injuries are 1.0 to 8.0%. The frequency of complications in patients with renal injury reaches 12% to 84%.[2]

Despite the diagnostic measures and therapeutic possibilities, disability and mortality among the injured with complications of combined kidney injury are still quite high. The urgency of the problem of diagnosing kidney damage in combined injury is determined by many factors. The most

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important of these include their severity, difficulty in diagnosis and treatment, and a high percentage of the mortality rate.[3,4] The highest difficulty is the diagnosis of renal complications, due to the blurring of clinical manifestations, the difficulty of interpreting the results obtained, and the limitations of a number of diagnostic investigations in patients with severe trauma. In the later periods of traumatic disease (TD), the condition of the victims allows, in most cases, carrying out the necessary examinations without significant restrictions, which in turn expands the possibilities for the timely diagnosis of complications.[2]

The X-ray examination traditionally starts from plain X-ray. However, in patients in serious condition, the required preparation before the examination is not carried out, which complicates the interpretation of plain radiographs and results of intravenous urography in cases of paresis of the colon or a decrease in renal excretory function on the affected side.[5] Ultrasound investigation (USI) is one of the modern, affordable methods for assessing the condition of the urinary tract (UT) and is carried out according to a standard protocol. USI has a number of undeniable advantages, such as not exposing the patient to radiation, accessibility, and non-invasiveness; but it also has significant disadvantages - limitation of visualization in obesity, flatulence, low sensitivity in visualizing the ureter, which reduces the diagnostic value of the method.[6]

Currently, a whole-body MSCT is the first-line diagnostic tool in patients with multiple injuries. In comparison with other radiation methods, computed tomography urography (CTU) has the greatest diagnostic value in determining the causes of hematuria and urinary obstruction.[7] MRI has a high potential for diagnosing acute renal and urinary tract injury in different periods of TD. MRI examination allows obtaining images of the urinary tract comparable in their informative value to MSCT in determining perirenal hematomas, assessing the depth of damage and viability of the renal parenchyma, and better visualizing of previously existing pathological changes in the organs of the urinary system.[8]

The aim of this study was to evaluate the effectiveness of MRI in diagnosing combined renal and ureteral injuries at different periods of TD.

Material and Methods

We analyzed the results of diagnostics and treatment of 139 patients (80 women and 59 men) with renal and ureteral injuries aged between 18 and 72 years. There were 67(48.2%) patients in the period of acute reaction to trauma, 40(28.8%) patients with early manifestations, and 32(23%) patients in the period of late manifestations. All patients with suspected renal and ureteric damage were examined and treated according to the standard scheme, depending on the nature of the dominant pathology.

In 127(91.4%) patients, an urgent plain abdominal X-ray was performed without any preliminary preparation, using the Axiom Luminos TF (Siemens). A plain abdominal X-ray in frontal projection was performed with the patient in standing position, or in cases where the patient could not stand behind the screen of the X-ray apparatus, the study was performed in a horizontal position. The radiation dose was of 0.033-0.21mSv.

USI of the abdominal and retroperitoneal space was performed in 108(77.7%) patients in the stage of the primary assessment of renal injury as it was a rapid non-invasive investigation. The standard time for complete examination of the kidneys and ureters was 15 minutes. Visualizing unexpanded ureters by USI was difficult; the quality of the investigation largely depended on the patient's physical constitution.

A whole-body MSCT was performed in 131(94.2%) patients on a GE Optima CT660 apparatus according to the "Polytrauma" program, using the nonionic contrast agents Ultravist (350mg I/ml) and Omnipaque (350mg I/ml). The radiation dose was of 10-18 mSv. The scanning area included: a) Skull and brain, cervical spine with the capture of the upper thoracic spine (up to the level of the ThIV vertebral body) – native scanning; b) Chest and abdominal cavities with intravenous contrast enhancement (with an assessment of the condition of internal organs, blood vessels, peripheral skeleton, and spine at the scan level). Unlike USI, MSCT is more independent of the operator; however, it involves ionizing radiation and requires intravenous injection of a contrast agent. If signs are detected of kidney injury (clinical or echographic) and stable hemodynamics, patients are recommended to perform the contrast-enhanced MSCT, but it requires puncture of the peripheral vein and multiphase scanning, taking approximately 10-15 minutes.

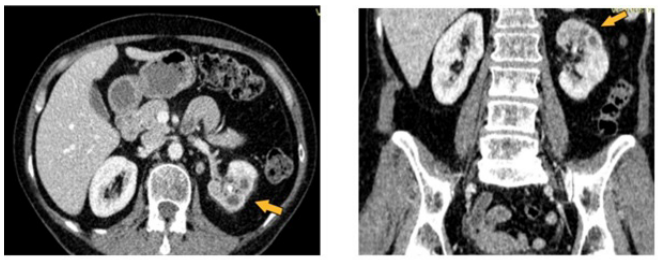
MRI has great potential for examining patients with UT injuries. MRI was performed in 125(89.9%) patients, including cases of pregnancy and a medical history of allergies. Image acquisitions were performed using Siemens Magnetom Harmony MRI Scanner. Each patient was scanned supine on the examination couch with his or her feet first, with a coil covering the diaphragm to iliac crest. The routine imaging protocol of the kidney included a coronal/sagittal T2 half Fourier single-shot turbo spin-echo sequence (HASTE) and a native coronal 3D gradient echo pulse T1-weighted (FLASH) sequence. In addition, the patients received a native, balanced steady-state free precession T2 mapping sequence (T2-prepared single-shot TrueFISP) in coronal plane, short inversion time inversion recovery (STIR) sequence, as well as DWI with ADC values.

Results and Discussion

All patients were divided into groups according to the type of renal and ureteric injury: combined renal injuries (125/89.9%), acute ureteral injuries (11/7.9%), and isolated kidney injury (3/2.2%). In case of damage, the clinical manifestations were nonspecific and the radiation signs were poor, which makes the diagnosis difficult. In 125(89.9%) patients, renal and ureteral damages were combined with other localizations: ribs and thoracic organs (42/33.6%); traumatic brain injury (30/24%); pectoral girdle bones, pelvic bones and free limbs (24/19.2%); the abdominal cavity organs (17/13.6%); spinal-cord injury (12/9.6%).

The greatest difficulty was renal damage in the acute and early periods of TD manifested by the blurred clinical image

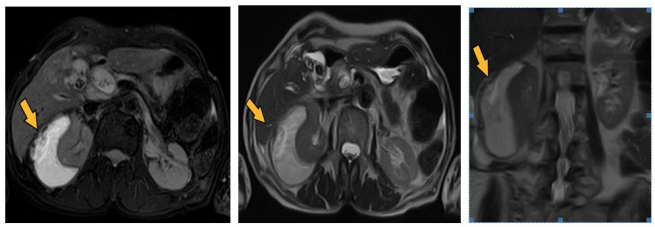
against a background of shock. Credible features of the kidney injuries were determined by the results of MSCT, which gave an opportunity to establish the severity of the injury (Fig. 1).



(a) (b)

Fig. 1. *Contrast-enhanced MSCT (a - b) of the abdominal cavity and retroperitoneal space (multiplanar reconstruction) of a 62-year-old patient on Day 4 after trauma: abscesses in the upper pole of the left kidney as a hypodense area with indistinct contours (arrows).*

MRI was performed in patients whose MSCT data were ambiguous and did not correspond to clinical and laboratory parameters. MRI has a high potential in the diagnosis of acute renal and urinary tract injury in different periods of TD and allows us to obtain images of the urinary tract comparable in its informational content to MSCT in determining the perirenal hematomas, assessing the depth of damage and viability of the renal parenchyma, providing better visualization of previously existing pathological changes of the urinary system organs while there is no radiation exposure to the patient and there is a natural tissue contrast without the need for the mandatory usage of intravenous contrast (Fig. 2).

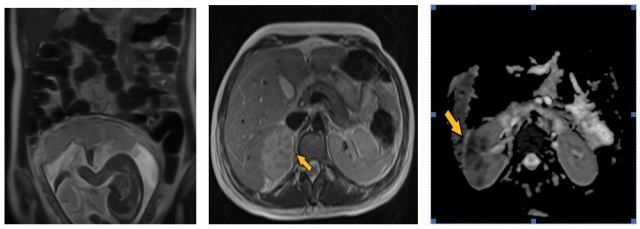


(a) (b) (c)

Fig. 2. *Native MRI of the abdominal cavity and retroperitoneal space (multiplanar reconstructions) of a 62-year-old patient on Day 4 after trauma: subcapsular hematoma in the upper, middle, and lower third of the right kidney (arrows).*

There were 26(20.3%) patients operated on in the acute period of TD. Nephrectomy was performed in 7(5.47%) cases with involvement of more than 25% of the kidney parenchyma in the pyo-inflammatory process; drainage of the retroperitoneal space - in 8(6.25%) cases; organ-preserving operations (wound closure or kidney resection) - in 11(8.6%) cases. Besides surgical interventions for the renal and ureteral injuries, there were indicated purulent complications for surgical treatment (48/37.5%).

Modern technologies and MRI software allowed us to eliminate many of the previous drawbacks connected to breathing artifacts. Optimizing protocols to reduce the total time of investigation to 10-15 min, using the necessary and sufficient program set in combination with the high differentiation of soft tissues, changed the place of MRI in the diagnostic algorithm in patients with renal damages. MRI has a high informational content and absence of ionizing radiation that makes this method demanded among pregnant women and the injured persons for whom contrast-enhanced MSCT is contraindicated (Fig.3). The MRI study revealed purulent inflammation of the kidneys in 3 pregnant patients who could not undergo CTU. MRI diagnostic viability is commeasurable with the contrast-enhanced MSCT. It doesn't have an ionizing impact and mostly does not need injection of any contrast agents. In our study, the duration of the investigation using a short protocol reached 15 min, which is similar to MSCT examination, taking to account the time for injection and catheterization of the peripheral vein, the native scanning, and multiphasic post-contrast scanning. The implementation of MRI allowed us to reduce radiation exposure and to increase the economic effectiveness of diagnosis by reducing the investigation costs. MRI, in combination with high indicators of efficiency and low costs and in contrast to intravenous contrast-enhanced MSCT, allows using this method for obtaining complete and sufficient information in the diagnosis of renal and ureteral injuries without significant restrictions (Fig. 4).



(a) (b) (c)

Fig. 3. *MRI of a pregnant woman (21-22 weeks): a) - T2-WI in the coronary plane, the uterus is enlarged according to the period of gestation, a single fetus in the cephalic presentation in the uterine cavity; b) MRU: thickening of the parenchyma with apostems in the cortical layer (arrows), c) DWI and ADC: foci of diffusion limitation correspond to multiple apostems (arrows).*

The developed short MRI protocol enables us to reach high diagnostic efficiency in patients with renal and ureteral injuries during different periods of TD and accurate detection of pathological changes in the abdominal cavity and retroperitoneal space. Taking into account the above mentioned, we calculated the scores of sensitivity (SE), specificity (SP), and accuracy (AC). The results presented in Table 1 showed that all the investigative methods demonstrate an increase in accuracy in detecting traumatic changes in the kidneys and ureters from the period of acute reaction to injury up to the period of late onsets of TD.

Conclusion

1. Contrast-enhanced MSCT has a high diagnostic efficiency in assessing complications in kidney and ureteral injuries at different periods of TD (accuracy of 89.2% for acute reaction, 88.8% for early manifestations, and 89.5% for late manifestations).

Table 1.

The diagnostic efficiency of the investigative methods in detecting renal and ureteral injuries at different periods of TD

Diagnostic methods	TB periods	Indicators of diagnostic efficiency,%		
		Se	Sp	Ac
Clinical and laboratory examination	acute reaction	61.2	56.1	57.5
	early onset	66.4	61.2	64.1
	late onset	86.3	82.7	85.6
Plain X-ray	acute reaction	-	-	-
	early onset	13.7	15.9	13.1
	late onset	17.8	44.7	25.2
USI	acute reaction	68.3	35.9	46.7
	early onset	71.9	64.7	72.0
	late onset	89.9	82.7	86.3
MSCT	acute reaction	92.0	84.9	89.2
	early onset	92.7	86.3	88.8
	late onset	94.2	84.8	89.5
MRI	acute reaction	-	-	-
	early onset	88.5	80.6	87.05
	late onset	92.1	87.8	89.9

2. MRI of the kidneys and ureters is indicated in periods of early and late manifestations of TD to detect renal complications in cases with a discrepancy between clinical manifestations and the results obtained by ultrasound and MSCT (accuracy of 87.5% for early manifestations and 89.9% for late manifestations).

3. The MRI protocol with both conventional spin-echo and STIR sequences allows achieving high diagnostic efficiency.

Competing Interests

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Women's Health

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The Genotype Distribution of Human Papillomavirus among HIV-Infected Women Planning Pregnancy in Irkutsk, Russia

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Abstract

The purpose of our research was to determine the detection frequency of 12 high-risk types of human papillomavirus (HPV) in women with human immunodeficiency virus (HIV) who are planning pregnancy and to assess the results of colposcopy and the state of the cervix in these women, depending on the presence of HPV.

***Methods and Results*:** We examined 31 women with HIV infection who sought pregnancy-planning advice at Scientific Center for Family Health and Human Reproduction Problems in Irkutsk during 2014-2015. All HIV-infected women were tested for the presence of high-risk HPV DNA in the epithelium of the cervical canal by PCR. Material for cytological examination (PAP test) was collected during gynecological examination. PAP tests were assessed according to the Bethesda system. All changes were divided into two types: ASCUS and SIL, the last was in its turn divided into two categories: LSIL and HSIL.

The frequency of HPV detection in HIV-infected women planning pregnancy was 71%. HPV 16 was found in 16(51.6%) HIV-infected women and ranked first in frequency among 12 types of HPV. HPV 33 and HPV 35 were found in 15(48.4%) and 12(38.7%) HIV-infected women, respectively, and ranked the second and third in frequency among 12 HPV types. We compared abnormal colposcopy results in two groups of patients with HIV infection: Group 1 (n=22) included women with HPV and Group 2 (n=9) included women without HPV. ASCUS was detected in all patients (100%) of Group 1 and in 6(66.7%) women of Group 2. Two women (9.1%) from Group 1 were diagnosed with LSIL and 10(45.5%) women with HSIL.

***Conclusion*:** HIV-infected women planning pregnancy and living in Irkutsk form a group at high-risk of HPV infection. (**International Journal of Biomedicine. 2021;11(3):346-350.**)

Key Words: human immunodeficiency virus • human papillomavirus • cervical cancer • cervical dysplasia

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Abbreviations

ASCUS, atypical squamous cells of undetermined significance; **HPV**, human papillomavirus; **HIV**, human immunodeficiency virus; **HSIL**, high-grade squamous intraepithelial lesions; **HAART**, highly active antiretroviral therapy; **LSIL**, low-grade squamous intraepithelial lesions; **PCR**, polymerase chain reaction; **SIL**, squamous intraepithelial lesions.

Introduction

Irkutsk region (Eastern Siberia) has an unfavorable HIV epidemiological situation among the regions of the Russian Federation. By the end of 2016, a total of 50,344 HIV-infected people had been reported, with 3185 new cases having predominantly sexual transmission—among them, 1445(45.4%) women with an average age of 30-39 years. HIV infection is spreading mainly among susceptible population groups: injection drug users, sex workers, men having sex with men, prisoners, and sexual partners of drug users.[1-3] However, recent studies have corroborated the active involvement of women of reproductive age in the HIV epidemic, which was found during examinations of pregnant women.[4-6]

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HIV infection progresses slowly, and its distinctive feature is the increasing suppression of the immune system functions, which contributes to the risk of opportunistic infections. Patients with HIV coinfection have more severe oxidative stress than HIV-monoinfected patients, which can contribute to the development of reproductive system disorders.[7,8] Among all the variety of opportunistic infections, sexually transmitted infections, including HPV, have the most aggravating effect on reproductive health.

HPV infection is one of the most widespread sexually transmitted infections.[9] On average, the frequency of HPV in the world is 10%. The highest frequency level of HPV is observed in Africa – 22.1%, and in Central America and Mexico – 20.4%. In North America, Europe, and Asia, the HPV frequency is 11.3%, 8.1% and 8.0%, respectively.[10] There are more than 100 types of HPV that can be found in wart tissues, condylomas and other tumors;[11] among them, about 40 types can be detected mainly in the anogenital region epithelium. HPV is divided into two groups: low- and high-risk types for cervical cancer development, and among them HPV type 16 has the greatest oncogenicity.

HPV infection among HIV-positive women is two times higher than in women without HIV infection.[12] At the same time, HPV infection in HIV-infected women has increased pathogenicity and significantly increases the risk of cervical lesions and cancer.[13] In HIV-infected women, it is important to assess the possible detrimental effect of opportunistic infections, including HPV, on the course of pregnancy and childbirth.[14]

The purpose of our research was to determine the detection frequency of 12 high-risk types of HPV in women with HIV who are planning pregnancy and to assess the results of colposcopy and the state of the cervix in these women, depending on the presence of HPV.

Materials and Methods

We examined 31 women with HIV infection who sought pregnancy-planning advice at Scientific Center for Family Health and Human Reproduction Problems in Irkutsk during 2014-2015. 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.

The general characteristics of women with HIV infection are presented in Table 1. The average age of the women was 30.9±4.5 years, ethnicity – Europeoids. Among 31 women, 22(71%) women had secondary special education, 9(29%) had secondary education, 7(22%) were married, 13(42%) had unregistered marriage, 2(6%) were single, and 9(30%) were divorced. Among 31 women, 22(71%) women had regular sex, 9(29%) women had irregular sex (less than 4 times per month); 19(61%) women used a condom, 9(29%) had interrupted sexual intercourse, 3(10%) did not use any contraception, and 20(64%) women had a permanent sexual partner with HIV infection.

Patients were diagnosed with HIV in the Irkutsk Regional AIDS Center. HIV stage 4-A was found in 13(42%) women, HIV stage 4-B in 18(58%). The average duration of HIV infection was 8±2.5 years. Fifteen(48%) patients were receiving HAART**.** HIV was mainly transmitted sexually in 80%, parenteral route of transmission of the virus was in 20%.

Each patient underwent colposcopy with 5% solution of acetic acid and 5% Lugol's iodine solution. We used the *CARL ZEISS E Colposcope* and the international classification of colposcopic terminology IFCPC Nomenclature (Rio de Janeiro, 2011). Material for cytological examination (PAP test) was collected during gynecological examination using vaginal specula. The impression smear was taken from the exocervix, from the border of the stratified epithelium and columnar epithelium of the cervical canal and from the lower third of the endocervix. The impression smear was obtained by scraping and was applied to the slide with a cervix brush. Further, the material was stained with hematoxylin and then with acid eosin. PAP tests were assessed according to the Bethesda system. All changes were divided into two types: ASCUS and SIL, the last was in its turn divided into two categories: LSIL and HSIL.

Table 1.

The general characteristics of women with HIV infection

Women	n=31
Age (years)	30.9±4.5
Education (%)	
Secondary special education	71
Secondary education	29
Marital status (%)	
Registered marriage	22
Unregistered marriage	42
in divorce	30
not married	6
Sexual relations (%)	
regularly	71
irregularly<4 times a month	29
Contraception (%)	
condom	61
interrupted sexual intercourse	29
did not use any contraception	10
Stage of HIV infection	
4-A	42
4-B	58
Average duration of HIV infection (years)	8±2.5
Regularly took highly active antiretroviral drugs HAART (%)	48
Way of HIV transmission (%)	
sexual way	80
parenteral route	20

All HIV-infected women were tested for the presence of high-risk HPV DNA in the epithelium of the cervical canal by PCR. We used reagents manufactured by the Central Research Institute of Epidemiology (Russia) and followed the manufacturer's instructions. Biological material was

sampled with the cervix brushes, which were placed in vials with transport medium (isotonic aqueous saline buffer solution with preservative). DNA was isolated from the obtained samples by sets of "DNA–Sorb–AM" reagents. We determined the presence of specific DNA segments of HPV by multiplex PCR in a thermocycler "Tertsik" (Russia), using a set of reagents "AmpliSens HPV HCR screen-Eph." Then HPV-positive samples were tested for the presence of 12 types of HPV (16,18,31,33,35,39,45,52,56,58,59,66) using a set of reagents "AmpliSens HPV genotype-Eph." The results of amplification reaction amplification were estimated by electrophoresis in 3% agarose gel, dyed with ethidium bromide.

Statistical analysis was performed using the statistical software STATISTICA (v10.0, StatSoft, USA). Baseline characteristics were summarized as frequencies and percentages for categorical variables and as means and SDs for continuous variables. Group comparisons with respect to categorical variables are performed using chi-square tests or, alternatively, Fisher's exact test when expected cell counts were less than 5; z-test was used to analyze the differences in proportions. A value of $P<0.05$ was considered significant.

Results

In the first stage, a screening study was conducted, which resulted in HPV detection in 22(71%) women. In the second stage, DNA samples in which HPV was detected were additionally typed to determine the HPV genotype. In the group of HPV-positive women, on average, each patient had three different types of HPV. One or two types of virus were found in 8(36.4%) women, three or four different types in 6(27.3%) women, five or seven types were also found in 6(27.3%) women, and more than seven types of virus in 2(9.1%) women.

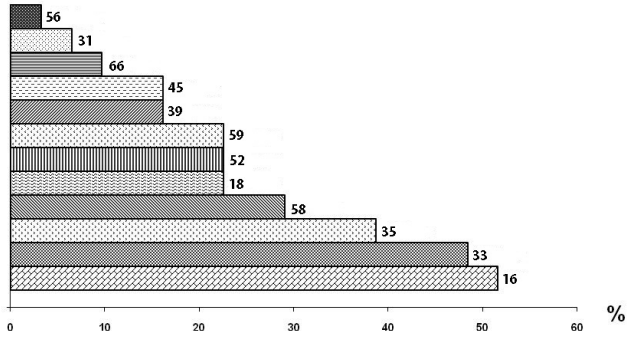

Fig. 1. *The frequency of 12 types of HPV in the group of women with HIV infection, %.*

HPV 16 was found in 16(51.6%) HIV-infected women and ranked first in frequency among 12 types of HPV (Figure 1). Only 2(12.5%) patients had HPV 16 mono-infection, and 14(87.5%) women had HPV 16 combined with other types of HPV. Most often, HPV 16 was combined with HPV 33 and HPV 35 - 12(75%) and 11(68.7%), respectively. Only 6(37.5%) patients with HPV 16 had combination with HPV 18. HPV 33 and HPV 35 were found in 15(48.4%) and 12(38.7%) HIV-infected women, respectively, and ranked the second and third in frequency among 12 HPV types. HPV 58 was detected in 9(29%) people and ranked the fourth; the detection rates of HPV 18 and HPV 52 were the same (in 7(22.6%) women), and they ranked fifth.

We compared abnormal colposcopy results in two groups of patients with HIV infection: Group 1 (n=22) included women with HPV and Group 2 (n=9) included women without HPV. ASCUS was detected in all patients (100%) of Group 1 and in 6(66.7%) women of Group 2. Two women (9.1%) from Group 1 were diagnosed with LSIL and 10(45.5%) women with HSIL.

Discussion

The frequency of HPV detection in HIV-infected women planning pregnancy was 71%. An epidemiological study previously conducted in Irkutsk showed that in the group of 641 HIV-infected women, the prevalence of HPV was 63.9%.[15] In Russia, similar data on the prevalence of HPV infection among HIV-infected people were obtained in St. Petersburg, where 113 women with HIV infection were examined, and HPV was detected in 80.5% of them.[16] Thus, the results of our study and other studies from other Russian regions demonstrate a high frequency of HPV in women with HIV infection.

The population-based study in Irkutsk (n=826) detected HPV in 37.7% of women without HIV infection.[17] The frequency of HPV is almost two times higher in HIV-infected women in our study ($\chi^2=12.59$; df=1; $P=0.001$).

Other authors confirm data on the higher frequency of HPV infection in groups of HIV-infected women. Shipulina et al.[18] compared the frequency of HPV between two groups of women with HIV infection (n=155) and without HIV (n=406) and showed that in the HIV group, HPV was detected in 38.7%, and in the group without HIV, HPV was found only in 14.8% of women. In the other study, a group of women (n=150) who were in prison were examined on the presence of HIV and HPV. It was shown that in the HIV group, HPV was detected in 58.2%, and in the group of women without HIV - only in 23%.[19] Thus, the results obtained in these studies showed that the prevalence of HPV infection in the group of HIV-infected women was 2.5 times higher than in the groups of women without HIV.

We found that HPV 16 ranks first among 12 types of high-risk-HPV. Our data are consistent with those of other authors on the high frequency of HPV 16 in the groups of HIV-infected women. In a study conducted in St. Petersburg, the frequency of HPV 16 in HIV-infected women was 39.8%. (16) In India, in HIV-infected women, HPV 16 was found in 58.5%, in Colombia in 46.3%, in Spain in 28% of cases.[20-22]

We compared the detection frequency of HPV 16 in women with HIV (our own data) and without HIV (literature data) living in Irkutsk (Eastern Siberia region). The frequency of HPV 16 type in Irkutsk was 33%.[17] The incidence of HPV 16 in the group of HIV-infected women in our study was 51.6%, which was 1.5 times higher (z=1.976; $P=0.048$).

A comparative analysis of the detection frequency of HPV types 33, 35 and 18 in groups of women with and without HIV living in Irkutsk showed some differences. The detection frequency of HPV 33 and HPV 35 in the group of HIV-infected women, according to the results of our study, was 48.4 and 38.7%, respectively. According to other authors, the frequency of detection of HPV 33 and HPV 35 among women aimed at HPV testing was 16.8 and 5.1%, respectively.[17] Statistically significant differences were obtained by comparing the frequency of HPV 33 and HPV 35 between these groups of women [(z=4.281; P<0.001) and (z=7.249; P<0.001), respectively]. The frequency of detection of HPV 18 in women with HIV, according to the results of our study, was 22.6%, and the frequency of detection of HPV 18 among women aimed at HPV testing was 6.3%.[17] Statistically significant differences were obtained by comparing the frequency of HPV18 between these groups of women (z=3.171; P=0,002).

Our study showed that after HPV 16, HPV33 and HPV 35 ranked the second and third, respectively, among 12 types of high-risk HPV. HPV 58 was the fourth, and both HPV 18 and 52 were the fifth. In other countries, according to the literature data, different types of HPV were in the second and third places after HPV 16 among HIV-infected women. So, in India, HPV 31 and HPV 56 were detected in 22.6% and 13.2% of HIV-positive women, respectively.[20] In Colombia, HPV 31 and HPV 18 were found in 32.9% and 30.6%, respectively.[21] In the Bahamas, HPV 18 was the first and it was found in 34.9%, HPV 58 and HPV 16 took the second and third places and were found in 30.2% and 27.9%, respectively.[23] In some countries, the frequency of HPV detection among HIV-infected women is slightly lower. Thus, in Korea, HPV 16 was detected in 10%,[24] and in Brazil only in 8% of HIV-positive women.[13]

Conclusion

The frequency of HPV detection was significantly higher in the group of HIV-infected women planning pregnancy than among women aimed at HPV testing and living in the same city (χ^2=12.59; df=1; P=0,001). HIV-positive women had HPV combined with several types; on average, they were infected with three different types of HPV. More than half of the women with HIV were infected with HPV 16, which has the greatest carcinogenic risk. HPV 33 and HPV 35 ranked second and third in the frequency of HPV detection, which were found in 48.4% and 38.7% of HIV-infected women, respectively. The frequency of detection of HPV 18 in our study was 22.6%. LSIL and HSIL were statistically significant more in HIV-infected women with HPV than in HIV-infected women without HPV (P=0.019).

HIV-infected women planning pregnancy and living in Irkutsk form a group at high-risk of HPV infection. It is necessary to monitor these patients for timely detection of HPV and for cervical screening.

Competing Interests

The authors declare that they have no competing interests.

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Epidemiology/Population Health

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Electrical Activity of the Heart in the Older Adults Suffering from Chronic Kidney Disease: Ethnic and Age Characteristics

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Abstract

The aim of the study was to investigate the ethnic and age-related characteristics of the electrical activity of the heart in older adults with chronic kidney disease (CKD).

Methods and Results: A total of 522 patients aged between 60 and 89 years with different stages of CKD were examined. For a comparative analysis, we formed 3 ethnic groups (Yakuts, Evens, and Russians) and 2 age groups (60-74 years and 75-89 years). All patients underwent ECG. The laboratory analysis included determining the blood creatinine level for further calculation of the GFR using the Cockcroft&Gault formula, followed by determining the stage of CKD. A decrease in renal function was more typical for people of 75-89 years. The results of the study confirmed the close relationship of CKD in older adults with the pathology of the cardiovascular system. Changes in the electrical activity of the heart predominated in Russians, compared to other ethnic groups, and were less pronounced in the Evens.(**International Journal of Biomedicine. 2021;11(3):351-354.**)

Key Words: ethnicity • older adults • electrical activity of the heart • chronic kidney disease

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Abbreviations

CKD, chronic kidney disease; **EAH**, electrical activity of the heart; **ECG**, electrocardiography; **GFR**, glomerular filtration rate; **LVH**, left ventricular hypertrophy.

Introduction

The high incidence of cardiovascular diseases in kidney pathology is well known.[1-3] On the one hand, pathology of the cardiovascular system, according to the results of numerous studies, is the main cause of death in patients with chronic kidney disease (CKD).[4-6] At the same time, in the older adults, the presence of a combination of two or more diseases often aggravates cardiovascular pathology.[7-9] Even mild renal dysfunction in patients with early stages of CKD can increase the risk of ischemic heart disease, myocardial infarction and other cardiovascular complications, significantly worsening the quality and prognosis of life in such patients.[10-12] In this regard, it is important to study the electrical activity of the heart and problems related to cardiorenal relationships in older adults suffering from CKD.

The aim of the study was to investigate the ethnic and age-related characteristics of the electrical activity of the heart (EAH) in older adults with CKD.

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Materials and Methods

A total of 522 patients aged between 60 and 89 years with different stages of CKD were examined. All patients were divided into 3 groups; their nationality was indicated by self-determination. Group 1 consisted of 174 patients - Evens living in the Arctic zone of Yakutia. Group 2 included 177 patients of Yakut nationality living in the Vilyui zone. Group 3 was comprised 171 patients of Russian nationality living in the Central and Vilyui zones of Yakutia.

The age of the examined patients varied from 60 to 89 years; the average age of the surveyed was 72.7±7.2 years (72.9±7.2 for men and 72.6±7.2 for women). The division by age groups was carried out on the basis of the WHO classification: the age group of 60-74 years, the age group of 75-89 years.

The research program included the following sections: informed consent of the respondent to conduct research and donate blood; a thorough and in-depth collection of medical history, review of outpatient medical records from clinics at the place of residence, clinical examination by an internist and nephrologist. All patients underwent ECG. The laboratory analysis included determining the blood creatinine level for further calculation of the GFR using the Cockcroft&Gault formula, followed by determining the stage of CKD. According to the classification of the KDIGO committee,[13] the stages of CKD, depending on the GFR, were distributed as follows: Stage 1 (≥90 ml/min/1.73m^2), Stage 2 (60-89 ml/min/1.73m^2), Stage 3A (45-59 ml/min/1.73m^2), and Stage 3B (30-44 ml/min/1.73m^2).

A standard 12-lead ECG was recorded at 50 mm/s for 10-12 cycles with a Cardiovit AG-I system (Austria). LVH was defined as a Sokolow-Lyon voltage amplitude of (SV1+RV5 or RV6) ≥35mV and a Cornell product of [(RaVL+SV3)×QRS duration] >2440 mV·ms.

Statistical analysis was performed using IBM SPSS Statistics for Windows, Version 19.0 (Armonk, NY: IBM Corp.). For descriptive analysis, results are presented as median (Me), first quartile (25th percentile) and third quartile (75th percentile). A non-parametric Kruskal-Wallis test was used for comparisons of median values among groups. Categorical variables were analyzed using the Chi-square test with the Yates' correction. A value of $P<0.05$ was considered significant.

The study was approved by the Ethics Committee of the Yakut Science Center of Complex Medical Problems.

Results and Discussion

Assessment of renal function showed that in both age groups, GFR was statistically significantly lower in Russians than in Yakuts and Evens (P=0.015) (Table 1). In the age group of 75-89 years, we found a decrease in renal function in all ethnic groups; statistically significant differences in age-related GFR were obtained in representatives of the Russian and Yakut nationalities (P<0.001). The data obtained is consistent with the studies of many foreign scientists on the effects of aging on kidney function.[14-16] Renal dysfunction is more common in the group of 75-89 years. Adult Russians of both age groups had an unfavorable background for dysfunction.

Table1.

GFR in each ethnic group, depending on the age category (ml/min /1.73m^2)

Age, years	Ethnos	Me	Q_1	Q_3
60-74 (n=265)	Evens (n=88)	78.5	61.5	91.75
	Yakuts (n=95)	82.0	68.0	90.0
	Russians (n=82)	69.0	46.5	83.75
75-89 (n=257)	Evens (n=86)	67.0	52.0	82.0
	Yakuts (n=82)	59.0	45.0	80.5
	Russians (n=89)	40.0	35.0	52.0

Ethnicity-dependent electrocardiographic changes in different age groups are presented in Table 2. In the group of 60-74 years, LVH was significantly more often recorded among the Yakuts than among compared the Russians (39.0%) and Evens (27.3%) (P=0.006). ECG changes, such as atrial enlargement, tachycardia, atrioventricular block, extrasystole, atrial fibrillation and pathologic Q waves, were observed more often among the Yakuts and Russians than among compared the Evens; however, the differences were statistically insignificant. In the group of 75-89 years, tachycardia was significantly more often detected in Russians (P=0.002); myocardial infarction was significantly less frequently recorded in Evens (12.8%) versus 29.3% in Yakuts and 33.7% in Russians (P=0.004). Other ECG changes did not have statistically significant differences.

A comparative analysis of combined pathology of the kidneys and the heart, depending on ethnicity and age, was carried out. Table 3 presents electrocardiographic changes, depending on the CKD stages for the age category of 60-74 years and each ethnic group. Among Evens of the age group of 60-74 years, ECG changes did not depend on renal function. Among the Yakuts, tachycardia was more often detected at Stage 3A; among Russians, extrasystoles were more often detected in Stage 3B.

Electrocardiographic changes registered in the age group of 75-89 years depending on the CKD stages are presented in Table 4. In Yakuts of the age group of 75-89 years, ECG changes did not depend on the CKD stage. Among Evens and Russians, atrial enlargement was more common in CKD Stage 3A (P<0.001). In Russians of the age group of 60-74 years with CKD Stage 3A, bradycardia was also most often registered (P<0.001).

In conclusion, a decrease in renal function is more typical for people of 75-89 years. The most severe kidney damage is experienced by representatives of the Russian nationality in the age group of 75-89 years. An electrocardiographic study of the state of the heart muscle showed that the frequency of the LVH, which is a sign of long-term arterial hypertension, increased as the stage of CKD progressed. The prevalence of tachycardia and extrasystole can be explained by an increase in the activity of the sympathetic nervous system in patients with CKD, especially when combined with chronic heart failure. Changes in the EAH predominate in Russians, compared to other ethnic groups, and are less pronounced in the Evens. The results of the study confirmed the close relationship of CKD in older adults with the pathology of the cardiovascular system.

Table 2.

Ethnicity-dependent electrocardiographic changes in different age groups

Age group	Ethnos	LVH		AE		B/c		T/c		AVB		E/s		AF		AQW	
		n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
60-74 (n=265)	Evens (n=88)	24	27.3	3	3.4	7	8.0	4	4.5	2	2.3	5	5.7	6	6.8	14	15.9
	Yakuts (n=95)	48	50.5	8	8.4	10	10.5	6	6.3	6	6.3	10	10.5	8	8.4	21	22.1
	Russians (n=82)	32	39.0	8	9.8	12	14.6	6	7.3	8	9.8	13	15.9	13	15.9	21	25.6
	χ^2	10.36		2.92		1.97		0.6		4.21		4.65		4.29		2.48	
	P	0.006		0.23		0.37		0.74		0.12		0.1		0.12		0.29	
75-89 (n=257)	Evens (n=86)	35	40.7	10	11.6	8	9.3	3	3.5	3	3.5	4	4.7	9	10.5	11	12.8
	Yakuts (n=82)	36	43.9	11	13.4	12	14.6	8	9.8	4	4.9	11	13.4	11	13.4	24	29.3
	Russians (n=89)	42	47.2	21	23.6	15	16.9	18	20.2	6	6.7	12	13.5	8	9.0	30	33.7
	χ^2	0.75		5.34		2.23		12.52		0.97		4.71		0.89		11.1	
	P	0.69		0.07		0.33		0.002		0.62		0.1		0.64		0.004	

LVH - left ventricular hypertrophy, AE - atrial enlargement, Bc - bradycardia, Tc- tachycardia, E/s - extrasystole, AF- atrial fibrillation, AQW - abnormal Q waves

*Ta**ble 3.***

Electrocardiographic changes, depending on the CKD stages for the age category of 60-74 years and each ethnic group

Ethnos	Stage of CKD	LVH		AE		B/c		T/c		AVB		E/s		AF		AQW	
		n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Evens (n=88)	1	7	24.1	0	0	1	3.4	2	6.9	2	6.9	3	10.3	5	17.2	2	6.9
	2	11	28.2	2	5.1	6	15.4	1	2.6	0	0	2	5.1	1	2.6	6	15.4
	3A	5	31.3	1	6.3	0	0	0	0	0	0	0	0	0	0	4	25.0
	3B	1	25.0	0	0	0	0	1	25	0	0	0	0	0	0	2	50.0
	χ^2	0.3		1.9		5.47		5.34		4.16		2.4		7.5		6.23	
	P	0.96		0.59		0.14		0.15		0.24		0.49		0.06		0.1	
Yakuts (n=95)	1	19	70.4	4	14.8	2	7.4	1	3.7	2	7.4	2	7.4	1	3.7	7	25.9
	2	21	38.9	4	7.4	7	13.0	2	3.7	2	3.7	7	13.0	5	9.3	11	20.4
	3A	6	60.0	0	0	1	10.0	3	30.0	1	10.0	0	0	1	10.0	3	30.0
	3B	2	50.0	0	0	0	0	0	0	1	25.0	1	25.0	1	25.0	0	0
	χ^2	7.54		2.79		1.09		10.68		3.27		2.69		2.29		1.82	
	P	0.06		0.43		0.78		0.014		0.35		0.44		0.52		0.61	
Russians (n=82)	1	2	13.3	1	6.7	2	13.3	2	13.3	1	6.7	1	6.7	1	6.7	4	26.7
	2	19	50.0	3	7.9	4	10.5	3	7.9	3	7.9	4	10.5	8	21.1	10	26.3
	3A	5	50.0	3	30.0	1	10.0	0	0	2	20.0	1	10.0	1	10.0	4	40.0
	3B	6	31.6	1	5.3	5	26.3	1	5.3	2	10.5	7	36.8	3	15.8	3	15.8
	χ^2	7.03		5.4		2.78		1.73		1.52		8.29		1.98		2.07	
	P	0.07		0.15		0.43		0.63		0.68		0.04		0.58		0.56	

LVH - left ventricular hypertrophy, AE - atrial enlargement, Bc – bradycardia, Tc- tachycardia, E/s – extrasystole, AF- atrial fibrillation, AQW - abnormal Q waves

Table 4.

Electrocardiographic changes, depending on the CKD stages for the age category of 75-89 years and each ethnic group

Ethnos	Stage of CKD	LVH		AE		B/c		T/c		AVB		E/s		AF		AQW	
		n	%	n	%	n	%	N	%	n	%	n	%	n	%	n	%
Evens (n=86)	1	7	77.8	0	0	1	11.1	1	11.1	0	0	1	11.1	0	0	2	22.2
	2	16	38.1	3	7.1	3	7.1	2	4.8	2	4.8	1	2.4	4	9.5	5	11.9
	3A	9	36.0	7	28.0	2	8.0	0	0	0	0	1	4.0	1	16.0	2	8.0
	3B	3	30.0	0	0	2	20.0	0	0	1	10.0	1	10.0	1	10.0	2	20.0
	χ^2	5.95		9.84		1.67		3.02		2.69		2.0		1.91		1.73	
	P	0.11		0.02		0.64		0.39		0.44		0.57		0.59		0.63	
Yakuts (n=82)	1	2	100	0	0	0	0	1	50.0	0	0	0	0	0	0	0	0
	2	18	47.4	2	5.3	4	10.5	3	7.9	1	2.6	4	10.5	4	10.5	12	31.6
	3A	10	41.7	5	20.8	5	20.8	2	8.3	1	4.2	5	20.8	5	20.8	7	29.2
	3B	6	33.3	4	22.2	3	16.7	2	11.1	2	11.1	2	11.1	2	11.1	5	27.8
	χ^2	3.61		4.82		1.65		3.92		2.05		1.8		1.8		0.95	
	P	0.31		0.19		0.65		0.27		0.56		0.61		0.61		0.82	
Russians (n=89)	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	2	5	62.5	0	0	0	0	2	25.0	1	12.5	1	12.5	0	0	2	25.0
	3A	17	56.7	15	50.0	13	43.3	4	13.3	2	6.7	4	13.3	3	10.0	15	50.0
	3B	20	39.2	6	11.8	2	3.9	12	23.5	3	5.9	7	13.7	5	9.8	13	25.5
	χ^2	3.14		18.03		22.72		1.34		0.48		0.01		0.87		5.38	
	P	0.21		<0.001		<0.001		0.51		0.79		0.99		0.65		0.07	

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ORIGINAL ARTICLE

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Genetic Predictors of Type 2 Diabetes in Yakuts

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Abstract

The goal of this study was to investigate the distribution of alleles and genotypes of the *KCNJ11* rs5219, *PPARG* rs1801282, *TCF7L2* rs7903146/rs12255372 SNPs in Yakuts with T2D, in comparison with other ethnic populations.

Methods and Results: The study cohort consisted of 26 Yakut patients diagnosed with T2D (YKT2D). Genotyping of rs5219 *(KCNJ11)*, rs1801282 *(PPARG)*, rs7903146 and rs12255372 *(TCF7L2)* SNPs was performed by pyrosequencing using PyroMark Q48 Autoprep sequencer (QIAGEN).

The genotyping of the studied group of Yakuts did not reveal statistically significant differences between control groups and YKT2D patients with respect to the polymorphic variants of the *KCNJ11*, *PPARG*, and *TCF7L2* genes. The allele frequency analysis of the polymorphisms of the *KCNJ11*, *PPARG*, and *TCF7L2* genes demonstrated a low frequency of the risk T-allele in the *TCF7L2* (rs7903146, rs12255372) in Asian populations, compared to other human populations. We identified three haplotypes [CG (90.5%), TT (6.8%), and TG (2.7%)] in the YKT2D cohort. Also, we observed a strong LD between two SNPs (rs7903146 and rs12255372) of the *TCF7L2* gene in the majority of groups, including YKT2D (D '= 1, LOD = 4.92), except for African populations, where a very weak LD (D '= 0.001-0.435, LOD = 0.0-0.73) was observed.**(International Journal of Biomedicine. 2021;11(3):355-360.)**

Key Words: *KCNJ11* • *PPARG* • *TCF7L2* • type 2 diabetes • single nucleotide polymorphism

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Abbreviations

GWAS, genome-wide association studies**; HWE**, Hardy-Weinberg equilibrium; **KATP,** adenosine triphosphate (ATP)−sensitive potassium channel; **KCNJ11**, potassium inwardly rectifying channel, subfamily J, member 11; **LD**, linkage disequilibrium; **PPARG,** peroxisome proliferator-activated receptor gamma; **SNPs**, single nucleotide polymorphisms; **T2D,** type 2 diabetes; **TCF7L2**, transcription factor-7–like 2.

Introduction

Type 2 diabetes (T2D) is characterized by insulin resistance and/or insufficient insulin production by β-cells.[(1)] T2D is believed to be a polygenic disorder that results from a complex interaction of many genes and environmental factors. SNPs are now well recognized as the most popular molecular markers for genetic studies. SNPs are the most common genetic variation; they occur, on average, once in every 400-1,000 base pairs along DNA.[(2-5)] To date, GWAS

have discovered >600 genetic variants associated with T2D.[6]

Multiple genes and their interactions are involved in the insulin secretion pathway. The glucose-dependent insulin secretion in β-cells of the pancreas is regulated by KATP. KATP is a heteromeric protein, composed of four inward-rectifier potassium ion channel (Kir6.2) tetramers, which form the pore of the KATP channel, as well as sulfonylurea receptor 1 subunits surrounding the pore. Kir6.2 is encoded by the *KCNJ11* gene—a member of the potassium channel genes. Closure of ATP-regulated K+ channels (KATP channels) plays a central role in glucose-stimulated insulin secretion in β-cells.[7] Numerous studies have reported the involvement of SNPs of the *KCNJ11* gene and their interactions in the susceptibility to diabetes. KCNJ11 rs5219 is a common variant in which substitution of C to T replaces glutamate with lysine at position 23 (E23K) in exon 1, causing a decrease of insulin secretion. Among the SNPs in the *KCNJ11* gene, rs5219 is associated with an increased risk for T2D in various populations.[7-9]

The *PPARG* gene is located on chromosome 3p25 and plays a critical role in adipose tissue formation and subcellular metabolism of arterial wall macrophage foam cells. *PPARG* controls insulin sensitivity by transcriptionally stimulating adipocyte-specific genes involved in insulin signaling, lipid accumulation, fatty acid uptake, and glucose uptake. The *PPARG* rs1801282C>G polymorphism, an SNP in exon 2 of PPAR-γ, encodes a proline→alanine substitution at amino acid residue 12. This mutation reduces the transcription of *PPARG*. The *PPARG* rs1801282C>G polymorphism has been extensively investigated and was found to be correlated with the risk of cardiovascular diseases and T2D.[10]

The *TCF7L2* gene is responsible for the synthesis of a transcription factor 7-like 2, which regulates the expression of the proglucagon gene and other genes involved in carbohydrate metabolism. The *TCF7L2* gene is localized on chromosome 10q25.3. The risk T-allele of the *TCF7L2* rs7903146 SNP is associated with increased *TCF7L2* expression, and decreased insulin content and secretion(11). Risk T-allele carriers are further characterized by an elevated plasma proinsulin level and an increased proinsulin-to-insulin ratio suggestive of perturbed proinsulin processing.[11-15] The rs12255372 SNP, located in the intron region of *TCF7L2*, contains a single base G to T transition at position 293. A few epidemiological studies have assessed the relationship between rs12255372 and T2D, but the results of these studies are contradictory.[16-21]

According to previous studies on the Yakut populations, the most significant variants involved in the development of T2D were identified in the *ABCC8* gene (rs1799859 SNP and rs10811661 SNP), *LPL* gene (Int6/PvuII G>A and Int8/Hind3 variants), and *RSTN* gene (CDKN2A/B rs34861192 SNP and rs32119177 SNP). There were no statistically significant differences between the control groups and patients with T2D with respect to the polymorphic variants of the *TCF7L2* and *KCNJ11* genes.[22]

The goal of this study was to investigate the distribution of alleles and genotypes of the *KCNJ11* rs5219, *PPARG* rs1801282, *TCF7L2* rs7903146/rs12255372 SNPs in Yakuts with T2D, in comparison with other ethnic populations.

Materials and Methods

The study cohort consisted of 26 Yakut patients diagnosed with T2D (YKT2D). Exclusion criteria were other types of diabetes, low fasting insulin levels, cancer, heart failure (NYHA class III-IV), concomitant corticosteroid or estrogen treatment, alcoholism, drug addiction, dementia, and serious mental disorders. The reference group was a cohort of different populations without T2D, obtained from the 1000 Genomes Project database and other sources.

Genotyping of rs5219 *(KCNJ11)*, rs1801282 *(PPARG)*, rs7903146 and rs12255372 *(TCF7L2)* SNPs was performed by pyrosequencing using PyroMark Q48 Autoprep sequencer (QIAGEN).

The study was approved by the Ethics Committee of the Center for Personalized Medicine at the Republican Clinical Hospital No. 3. Written informed consent was obtained from each research participant (or the participant's parent/guardian).

Statistical analysis was performed using Microsoft Excel 2010.and PASW Statistics 18. The correspondence of the distributions of genotypes to the expected values at HWE and comparison of the frequencies of allelic variants/genotypes were performed using the chi-square test. Haploview software (ver. 4.2) was used to assess the *TCF7L2* haplotypes and frequencies based on genotyping data and to test the association between alleles and haplotypes of the *TCF7L2* gene.[23] A probability value of $P<0.05$ was considered statistically significant.

Results and Discussion

The results of this study show that the distribution of genotypes for all studied SNPs do not have significant deviations from the HWE. Comparative analysis of allele and genotype frequency distribution in the examined group is presented in Table 1.

Table 1.

Distribution of alleles and genotypes of polymorphisms of genes KCNJ11, PPARG, and TCF7L2 in a group of Yakut patients with T2D

Gene (RefSNP)	Dis	Genotype			Alleles		χ^2	P
KCNJ11 (rs5219)		CC	CT	TT	C	T		
	OF	42.3	46.2	11.5	0.654	0.346	0.010	0.920
	EF	42.8	45.3	12.0				
PPARG (rs1801282)		CC	CG	GG	C	G	χ^2	P
	OF	65.4	30.8	3.8	0.808	0.192	0.002	0.961
	EF	65.2	31.1	3.7				
TCF7L2 (rs7903146)		CC	CT	TT	C	T	χ^2	P
	OF	92.3	7.7	0.0	0.962	0.038	0.042	0.838
	EF	92.5	7.4	0.1				
TCF7L2 (rs12255372)		GG	GT	TT	G	T	χ^2	P
	OF	96.15	3.85	0.00	0.981	0.019	0.010	0.920
	EF	96.19	3.77	0.04				

Dis- distribution, OF- observed frequency, EF- expected frequency

An analysis of the frequency distribution of alleles and genotypes of the *KCNJ11* rs5219 SNP in a sample of YKT2D revealed the predominance of the heterozygous CT genotype (46.2%); the TT genotype (11.5%) was less common. Several meta-analyses and association studies reported a strong association between the r5219 (*KCNJ11 E23K*) and susceptibility to T2D, mainly in Caucasians and in some Asian populations.[24-26] However, some other association studies did not show any association between this polymorphism and susceptibility to T2D.[27]

The study of the *PPARG* rs1801282 showed the prevalence of the CC genotype (65.4%), while the frequency of the GG genotype was 3.8%. The *PPARG* rs1801282C>G polymorphism has been extensively studied and found to correlate with the risk of cardiovascular disease and T2D.[28-31] However, other studies reported contradictory results.[32] For example, it was shown that the Pro12Ala polymorphism protects against diabetes in Caucasians, but not in the South Asian population.[33-34]

The *TCF7L2* rs7903146 SNP was characterized by the prevalence of the CC genotype (92.3%), while the TT

Table 2.

Frequency of risk alleles in KCNJ11, PPARG and TCF7L2 gene polymorphisms in the cohort of Yakuts with T2D and other ethnic populations

Group	Genes and polymorphisms				Reference
	KCNJ11	*PPARG*	*TCF7L2*		
	rs5219	rs1801282	rs7903146	rs12255372	
	T	G	T	T	
YKT2D	34.6 (26)	19.2 (26)	3.8 (26)	1.9 (26)	-
YKTH	33 (348)	15 (348)	5 (348)	Without data	[35]
RUS	43.9 (264)	20.7 (94)	27.6 (201)	16.1 (597)	[27-40]
KGZ	33 (109)	13 (109)	11 (109)	Without data	[36]
JPN	33 (104)	3 (104)	3 (104)	2 (104)	[23]
CDX	22.6 (93)	0.5 (93)	2.2 (93)	1.1 (93)	
GBR	26.4 (48)	12.1 (22)	25.8 (47)	26.4 (48)	
IBS	38.3 (82)	11.7 (22)	39.7 (85)	37.4 (80)	
PEL	32 (85)	26 (85)	14 (85)	11 (85)	
PJL	44 (96)	14 (96)	25 (96)	21 (96)	
STU	30.4 (62)	12.3 (25)	33.8 (69)	22.1 (45)	
MSL	0	0	22.9 (39)	38.8 (66)	
YRI	0	0	24.1 (52)	30.1 (65)	
ACB	5.7 (11)	1.6 (3)	27.6(53)	24.5 (47)	
ASW	13.9 (17)	2.5 (3)	36.1 (44)	27/9 (34)	
ESN	0	0	24.7 (49)	29.3 (58)	

Abbreviations: YKT2D - Yakuts patients with T2D; YKTH - healthy Yakuts without T2D; RUS - healthy Russians; KGZ - healthy Kyrgyz; ACB - African Caribbean in Barbados; ASW - African Ancestry in Southwest US; CDX - Chinese Dai in Xishuangbanna, China; ESN - Esan in Nigeria; GBR - British in England and Scotland; GWD - Gambian in Western Division, The Gambia; IBS - Iberian populations in Spain; JPT - Japanese in Tokyo, Japan; MSL - Mende in Sierra Leone; MXL - Mexican Ancestry in Los Angeles, California; PEL - Peruvian in Lima, Peru; PJL - Punjabi in Lahore, Pakistan; STU - Sri Lankan Tamil in the UK; YRI - Yoruba in Ibadan, Nigeria.

Table 3.

The frequency distribution of TCF7L2 gene haplotypes for two SNPs (rs7903146 and rs12255372) in Yakuts with T2D and other ethnic populations

Group	Haplotype				Linkage disequilibrium (LD)		Reference
	CG	CT	TT	TG	D'	LOD	
YKT2D	90.5	-	6.8	2.7	1.0	4.92	-
JPN	96.6	0.5	1.9	1.0	0.793	5.24	[23]
CDX	97.8	-	1.1	1.1	1.0	2.99	
GBR	73.1	1.1	25.3	0.6	0.971	26.94	
IBS	58.8	1.5	35.0	4.7	0.933	27.02	
PEL	86.5	-	10.6	2.9	1.0	14.89	
MXL	76.5	1.6	17.9	4.0	0.893	12.11	
PJL	74.5	0.5	20.8	4.2	0.966	21.95	
STU	65.0	0.11	20.9	12.9	0.923	13.77	
MSL	49.9	27.2	11.6	11.3	0.193	0.26	
YRI	50.9	25.0	5.1	19.0	0.296	0.27	
ACB	54.7	17.7	6.8	20.8	0.001	0.0	
ASW	45.7	18.2	9.7	26.4	0.039	0.0	
ESN	50.1	25.2	0.41	20.7	0.435	0.73	
GWD	50.5	26.5	10.2	12.8	0.119	0.22	

Abbreviations: (see Table 2).

genotype was not found in the studied group. The *TCF7L2* rs12255372 was characterized by the predominance of the GG genotype (96.1%) and the absence of the TT genotype.

The comparative analysis of risk allele frequencies in *KCNJ11, PPARG,* and *TCF7L2* gene polymorphisms in the studied group and other ethnic populations is presented in Table 2.

The allele frequency analysis showed a low frequency of the risk T-allele of the *TCF7L2* (rs7903146 and rs12255372) SNPs in Asian populations, compared to other ethnic groups (Yakuts without T2D - 3.8% and 1.9%, Kyrgyz - 11%, Japanese - 3% and 2%, Han Chinese from southern regions of China - 2.2% and 1.1%). The frequency of the risk T allele of the *TCF7L2* rs7903146 SNP (3.8%) in the studied YKT2D cohort was similar to the frequency of the T allele in Yakuts without T2D (5%). Probably, the low frequency of the risk T allele in rs7903146, rs12255372 SNPs in Asian populations contributes to the low incidence of T2D.

There was a strong LD between two SNPs (rs7903146 and rs12255372) of the *TCF*7L2 gene (Fig. 1 and Table 3) in almost all groups, including YKT2D (D '= 1, LOD = 4.92), except for African populations, where a very weak LD (D '= 0.001-0.435, LOD = 0.0-0.73) was observed. This is likely due to a high genetic diversity in African populations. This can also indicate that the ancient humans that left the African continent and settled in Europe, Asia, and America went through a strong population decline, the so-called "bottleneck." And our results can indirectly confirm that the division of the human race into Mongoloid and Caucasian races occurred after the exodus from Africa.

The frequency distribution of *TCF7L2* gene haplotypes for two SNPs (rs7903146 and rs12255372) based on all detected variants is presented in Table 3. There are four possible haplotypes CG, CT, TT, and TG for rs7903146 and rs12255372 polymorphisms. We were able to identify three haplotypes [CG(90.5%), TT(6.8%), and TG(2.7%)] in the studied YKT2D group. These haplotypes were also found in the Chinese population and Peruvians. A similar haplotype frequency was observed in Peruvians [CG(86.5%), TT(10.6%), and TG(2.9%)]. We also observed a low frequency of haplotypes with the risk T allele in Yakuts, Chinese and Japanese people, and the absence of a heterozygous CT haplotype in Yakuts and Chinese (Table 3).

In conclusion, the genotyping of the studied group of Yakuts did not reveal statistically significant differences between control groups and YKT2D patients with respect to the polymorphic variants of the *KCNJ11, PPARG,* and *TCF7L2* genes. The allele frequency analysis of the polymorphisms of the *KCNJ11, PPARG,* and *TCF7L2* genes demonstrated a low frequency of the risk T-allele in the *TCF7L2* (rs7903146, rs12255372) in Asian populations, compared to other human populations. We identified three haplotypes [CG(90.5%), TT(6.8%), and TG(2.7%)] in the YKT2D cohort. Also, we observed a strong LD between two SNPs (rs7903146 and rs12255372) of the *TCF7L2* gene in the majority of groups, including YKT2D (D '= 1, LOD = 4.92), except for African populations, where a very weak LD (D '= 0.001-0.435, LOD = 0.0-0.73) was observed.

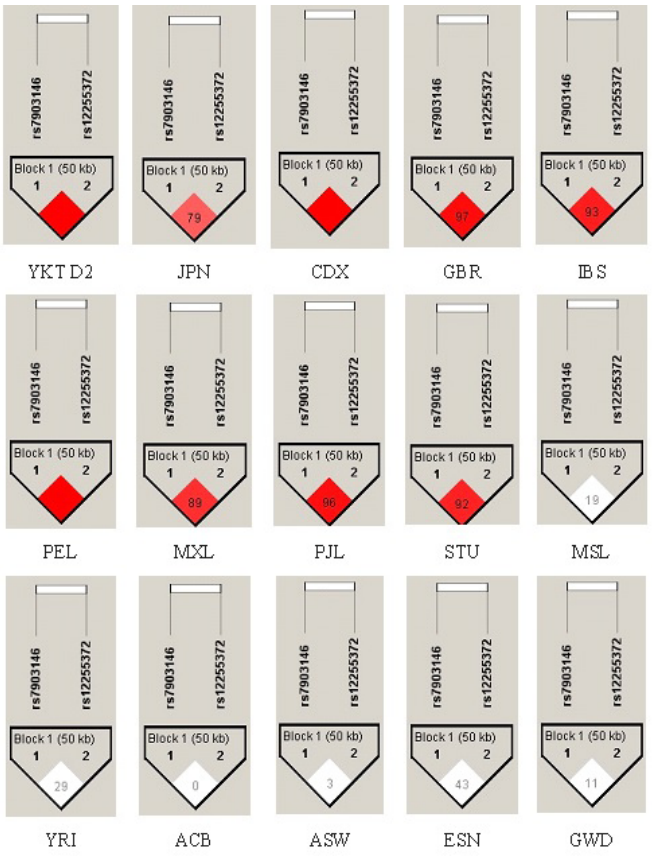

Fig. 1. *LD between the TCF7L2 (rs7903146, rs12255372) SNPs*

The color scheme shows the strength of adhesion between SNPs: bright red – a strong link (D'=1, LOD=2), pink-red – a significant link (D'<1, LOD=2), white – poor link (D'<1, LOD<2).

Abbreviations: YKT2D - Yakuts patients with T2D; ACB - African Caribbean in Barbados; ASW - African Ancestry in Southwest US; CDX - Chinese Dai in Xishuangbanna, China; ESN - Esan in Nigeria; GBR - British in England and Scotland; GWD - Gambian in Western Division, The Gambia; IBS - Iberian populations in Spain; JPT - Japanese in Tokyo, Japan; MSL - Mende in Sierra Leone; MXL - Mexican Ancestry in Los Angeles, California; PEL - Peruvian in Lima, Peru; PJL - Punjabi in Lahore, Pakistan; STU - Sri Lankan Tamil in the UK; YRI - Yoruba in Ibadan, Nigeria.

Competing Interests

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

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Association of the *AGTR1* Gene A1166C (rs5186) Polymorphism with Essential Hypertension in the Indigenous Population of the Arctic

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Abstract

The research objective was to study the association of the AGTR1 rs5186 SNP (the A1166C variant) with essential hypertension among indigenous people of the Arctic territory of Yakutia.

Methods and Results: A total of 351 participants (224 women and 127 men) were examined, including 56 Yakuts, 34 Chukchi, 77 Yukaghirs, and 184 Evens. The Case (n=168) and Control (n=183) groups were formed. Allelic variants of the AGTR1 rs5186 SNP were tested by real-time PCR. We did not find statistically significant differences in the frequency distribution of the alleles and genotypes of the AGTR1 rs5186 SNP between the Case group and the Control group.

Conclusion: The obtained data show no association of the AGTR1 A1166C polymorphism with EHT in the representatives of indigenous people of the Arctic territory of Yakutia.(**International Journal of Biomedicine. 2021;11(3):361-366.**)

Key Words: essential hypertension • genotype • *AGTR1* gene • A1166C • rs5186 • Yakutia

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Abbreviations

AGTR1, angiotensin II type I receptor; **AO**, abdominal obesity; A**H**, arterial hypertension; **BP**, blood pressure; **DBP**, diastolic BP; **EHT**, essential hypertension; **FPG,** fasting plasma glucose; **GWAS**, genome-wide association studies; **HDL-C**, high-density lipoprotein cholesterol; **HWE**, Hardy-Weinberg equilibrium; **LDL-C**, low-density lipoprotein cholesterol; **RAAS**, renin–angiotensin–aldosterone system; **SBP**, systolic BP; **TC**, total cholesterol; **TG**, triglycerides; **WC**, waist circumference.

Introduction

Arterial hypertension (AH) is still one of the most common problems in cardiology and is responsible for high cardiovascular morbidity and mortality. Essential hypertension (EHT) is a multifactorial disease with a complex genetic basis. Large-scale GWAS have successfully established ~800 genetic loci for SBP, DBP, and hypertension in multiple ethnic groups.[1] Several reviews and meta-analyses summarize the vast number of studies of the RAAS genes in cardiovascular physiology, disease, and treatment.[2-4] The RAAS plays a fundamental role in blood pressure maintenance and is implicated in the pathogenesis of hypertension. However, the results of the gene studies of individual RAAS candidates vary depending on population or ethnicity. Among the great number of gene candidates encoding the RAAS components, the *AT1R* gene is of great interest. Polymorphisms within this gene have

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been extensively studied in association with hypertension; however, findings are conflicting.[5]

The most well-studied of the *AGTR1* SNP is rs5186 (also termed the A1166C variant) located in the 3′ UTR. Frequencies of this variant range from 0.19–0.31 in populations of European descent,[6-9] 0.03–0.11 in populations of Asian descent,[10-13] and 0.05–0.08 in studies of populations of African descent. Numerous studies have been published associating polymorphisms of the *AGTR1* gene with AH; however, results have been inconsistent.[5,17,18]

It has been hypothesized that the *AGTR1* rs5186 SNP, an AC nucleotide substitution at position 1166 in the 3′ untranslated region of chromosome 3, may affect mRNA-155 stability and transcription. In some populations, a link between being a carrier of the C allele rs5186 and an increased risk of developing essential hypertension has been demonstrated.[17,18]

The Republic of Sakha (Yakutia) (RS(Y)) is a region where extreme climatic factors have a depleting effect on the functional reserves of the human body. The tension of the adaptive mechanisms often manifests itself in the form of an increase in BP. Changes in diet and physical activity have led to widespread overweight and obesity among indigenous populations of the North, which also contributes to an increase in BP.[19] Under these conditions, the search for genetic markers of predisposition to the development of hypertension is of both scientific and practical interest

Our research objective was to study the association of the *AGTR1* rs5186 SNP (the A1166C variant) with EHT among indigenous people of the Arctic territory of Yakutia.

Materials and Methods

A one-stage epidemiological study was carried out in the Arctic territory of the RS(Y). The Case (EHT+) and Control (EHT-) groups were formed. A total of 351 participants (224 women and 127 men) were examined, including 56 Yakuts, 34 Chukchi, 77 Yukaghirs, and 184 Evens. The average age was 45.9±12.5 years. Nationality was determined through participants' self-identification.

The study was approved by the Ethics Committee of the Yakut Science Center of Complex Medical Problems. Written informed consent was obtained from each patient.

Inclusion criteria for the Case group (n=168) were representatives with EHA of indigenous peoples of Yakutia (the Yakuts, the Evens, the Chukchi, the Yukaghir), and being 18 years and older. Inclusion criteria for the Control group (n=183) were representatives without EHT of indigenous peoples of Yakutia (the Yakuts, the Evens, the Chukchi, the Yukaghir), and being 18 years and older. Exclusion criteria were representatives of non-indigenous nationality and those with secondary hypertension.

The research program included the following sections: a questionnaire for objective assessment of state; informed consent of the respondent to conduct research and donate blood; anthropometric examination; and blood sampling from the cubital vein in the morning on an empty stomach, with 12-hour abstinence from food.

Abdominal obesity (AO) was confirmed at WC ≥ 94 cm in males and ≥ 80 cm in females (VNOK, 2009).

Blood pressure (BP) was measured twice with an OMRON M2 Basic automatic tonometer, with subjects in a sitting position. Average BP was calculated with a margin of permissible measurement error of ±3 mmHg, according to the instructions for the correct measurement of BP outlined in the European clinical guidelines for the diagnosis and treatment of hypertension. The diagnosis of AH was based on 2017 ACC/AHA Guideline for or the Prevention, Detection, Evaluation, and Management of High Blood Pressure in Adults.[20]

Laboratory methods of the research included the assessment of FPG and blood levels of TG, HDL-C, and LDL-C. Lipid metabolism disorders were diagnosed according to the Russian national recommendations of the VII revision(the Russian Society of Cardiologists [RSC, 2020]), considering the European recommendations (2019): TC >5,0 mmol/l; TG>1.7 mmol/l; HDL-C <1.0 mmol/l in males and <1.2 mmol/l in females; LDL-C >3.0 mmol/l. The atherogenic index (IA) was determined by the formula: IA=(TC-HDL-C)/HDL-C (Klimov AN, Nikulcheva NG, 1999). Impaired fasting glucose was defined as FPG level >5.6 mmol/l. Respondents with these disorders also included participants receiving specific medication for these conditions.

Genotyping of *the AGTR1* rs5186 SNP (the A1166C variant)

Genomic DNA was isolated from the peripheral blood leukocytes using a standard phenol–chloroform extraction technique. Allelic variants of the AGTR1 rs5186 SNP_were tested by real-time PCR on the «Real-time CFX96» amplifier (BioRad, USA) using Lytech kits (Lytech R&D LLC, Moscow) in accordance with the manufacturer's instructions. For quality control, 10% of samples were randomly repeated, with complete congruence.

Statistical analysis was performed using IBM SPSS Statistics for Windows, Version 22.0 (Armonk, NY: IBM Corp.). Baseline characteristics were summarized as frequencies and percentages for categorical variables and as median (interquartile range (IQR; 25th to 75th percentiles) for continuous variables. Mann-Whitney U test and Kruskal-Wallis test were used, respectively, to compare differences between 2 and 3 or more independent groups. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated. Deviation from Hardy-Weinberg equilibrium and differences in allele distributions between the two groups were assessed by χ^2- test with 1 degree of freedom (df). A probability value of $P<0.05$ was considered statistically significant.

Results and Discussion

The frequencies of the AA, AC and CC genotypes of the *AGTR1* rs5186 SNP (the A1166C variant) in the groups of Yakuts, Evens, and Yukaghirs corresponded to the HWE. In the Chukchi group, which was represented by 34 participants, the distribution of the genotype frequency was not in the HWE (Table 1).

The frequency of the C allele varied from 0.13 in the Evens to 0.35 in the Chukchi. According to the ALFA (Allele

Frequency Aggregator) project, the frequency of the C allele carriage averages 0.28 (n=238604), varying, depending on population, from 0.009 among Africans (n=354) to 0.30 among Hispanics (n=6874). Among the populations of Southeast Asia, the prevalence of the C allele is 0.08–0.09. [21] Thus, according to the presented study, the frequency of the C allele in the indigenous ethnic groups of Yakutia is, on average, higher than in the populations of Southeast Asia and Africa.

We did not find statistically significant differences in the frequency distribution of the alleles and genotypes of the *AGTR1* rs5186 SNP between the Case group and the Control group (Table 2).

In research literature, the data on the link between the AGTR1 rs5186 SNP and essential hypertension is contradictory.[17,18,22-25] Regarding China, a comparison of three genetically different populations with significant differences in the prevalence of EHT suggested that the A allele may

Table 1.

The frequency distribution of the alleles and genotypes of the AGTR1 rs5186 SNP (the A1166C variant) in the groups of Yakuts. Evens. and Yukaghirs

Allele /Genotype	Indicator	Yakuts (n=56)	Evens (n=184)	Chukchi (n=34)	Yukaghirs (n=77)	Total (n=351)
A	Total	90	321	44	116	571
	Frequency (95% CI)	80.4 (71.5-87.2)	87.2 (83.3-90.4)	64.7 (51.9-75.9)	80.6 (72.9-86.6)	81.3 (78.3-84.1)
C	Total	22	47	24	38	131
	Frequency (95% CI)	19.6 (12.8-28.5)	12.8 (9.6-16.7)	35.3 (24.1-48.1)	26.4 (19.5-34.5)	18.7 (15.9-21.7)
AA	Total	36	140	10	43	229
	Frequency (95% CI)	64.3 (50.0-76.7)	76.1 (69.2-81.9)	29.4 (14.5-48.5)	55.8 (44.0-67.1)	65.2 (60.0-70.1)
AC	Total	18	41	24	30	113
	Frequency (95% CI)	32.1 (20.2-46.4)	22.3 (16.6-29.1)	70.6 (51.5-85.5)	38.9 (28.1-50.9)	32.2 (27.4-37.3)
CC	Total	2	3	0	4	9
	Frequency (95% CI)	3.6 (0-14.3)	1.6 (0-5.3)	0	5.2 (0.7-14.0)	2.6 (1.1-5.0)
χ^2 (HWE)		0.019	8.08	10.1	0.179	1.284
P-value		0.892	0.99	0.001	0.673	0.257

Table 2.

The frequency distribution of the alleles and genotypes of the AGTR1 rs5186 SNP (the A1166C variant) between the Case group and the Control group

Ethnos	Group	Allele/Genotype, n (%)			OR (95% CI); *P*-value
		A	C		
Yakuts	EHT-	31 (77.5)	9 (22.5)		0.76 (0.29-1.97); *P*=0.750
	EHT+	59 (81.9)	13 (18.1)		
Evens	EHT-	176 (87.1)	26 (12.9)		0.98 (0.53-1.8); *P*=1.0
	EHT+	145 (87.3)	21 (12.7)		
Chukchi	EHT-	29 (65.9)	15 (34.1)		1.16 (0.41-3.27); *P*=0.988
	EHT+	15 (62.5)	9 (37.5)		
Yukaghirs	EHT-	59 (73.8)	21 (2.2)		0.84 (0.40-1.75); *P*=0.776
	EHT+	57 (77.0)	17 (23.0)		
All groups	EHT -	295 (80.6)	71 (19.4)		0.90 (0.62-1.32); *P*=0.600
	EHT+	276 (82.1)	60 (17.9)		
		AA	AC	CC	
Yakuts	EHT-	12 (60)	7 (35.0)	1 (5.0)	AA and AC: 0.79 (0.24-2.54); *P*=0.920 AA and CC: 0.50 (0.03-8.71); *P*=1.0 AA and AC+CC: 0.75 (0.24-2.33); *P*=0.835
	EHT+	24 (66.7)	11 (30.6)	1 (2.8)	
Evens	EHT-	77 (76.2)	22 (21.8)	2 (2.0)	AA and AC: 1.06 (0.53-2.12); *P*=1.0 AA and CC: 0.61 (0.05-6.89); *P*=1.0 AA and AC+CC: 1.02 (0.52-2.0); *P*=1.0
	EHT+	63 (75.9)	19 (22.9)	1 (1.2)	
Chukchi	EHT-	7 (31.8)	15 (68.2)		AA and AC: 1.40 (0.29-6.83); *P*=0.982
	EHT+	3 (25.0)	9 (72.0)		
Yukaghirs	EHT-	22 (55.0)	15 (37.5)	3 (7.5)	AA and AC: 1.05 (0.41-2.66); *P*=0.922 AA and CC: 0.35 (0.03-3.63); *P*=0.696 AA and AC+CC: 0.93 (0.38-2.29); *P*=1.0
	EHT+	21 (56.8)	15 (40.5)	1 (2.7)	
Total	EHT-	118 (64.5)	59 (32.2)	6 (3.3)	AA and AC: 0.97 (0.62-1.55); *P*=0.997 AA and CC: 0.53 (0.13-2.18); *P*=0.581 AA and AC+CC: 0.93 (0.60-1.45); *P*=0.841
	EHT+	111 (66.1)	54 (32.1)	3 (1.8)	

be a predisposing factor for EHT in Tibetan men, while no association was found in the other two populations.[22] In a case-control study conducted in Poland (250 patients with stable EHT and 150 individuals with normal BP), the C allele and CC genotype were statistically significantly more frequent in patients with hypertension.[25] In a similar study conducted in India, individuals with the CC genotype were 2.4 times more likely to develop EHT (P=0.0001) than individuals with the AC and AA genotypes.[23] At the same time, in the study by Suita, conducted in Japan, involving 1492 hypertensive subjects and 2426 normotensive subjects, no association was found between the A1166C variants of the *AGTR1* gene and hypertension.[24] Similar results were obtained by researchers in Tunisia.[26]

The *AGTR1* A1166C polymorphism was found to be linked to the presence and severity of nonalcoholic fatty liver disease, nonalcoholic steatohepatitis, liver fibrosis, dyslipidemia, insulin resistance, and metabolic syndrome.[27-30] Considering the above data, in our analysis, the carriers of the A allele and C allele were compared in terms of the level of metabolic parameters (Table 3). The carriers of different alleles and genotypes were comparable in age and gender structure. We did not find any differences in BP levels between carriers of different genotypes. It should be noted that the levels of SBP and DBP were compared for all study participants, including those taking antihypertensive drugs, which could change the results obtained.

Table 3.

Comparison of age and metabolic parameters in carriers of different alleles and genotypes of the A1166C polymorphism of the AT1R gene

Indicator	Me (Q_1-Q_3)			*P*-value
Allele				
	A		C	
Age, years	48.0 (36.0-55.0)		47.0 (35.0-55.0)	0.987
WC, cm	88.0 (78.0-98.0)		83.0 (35.0-98.0)	0.044
SBP, mmHg	130.0 (120.0-150.0)		130.0 (35.0-150.0)	0.337
DBP, mmHg	80.0 (80.0-90.0)		80.0 (35.0-90.0)	0.347
FPG, mmol/	4.4 (3.9-5.0)		4.2 (35.9-5.0)	0.099
TG, mmol/l	1.0 (0.7-1.4)		0.9 (35.7-1.4)	0.129
TC, mmol/l	4.9 (4.4-5.5)		4.9 (35.4-5.5)	0.385
HDL-C, mmol/l	1.2 (1.0-1.5)		1.4 (35.0-1.5)	0.011
LDL-C, mmol/l	3.2 (2.7-3.7)		3.0 (35.7-3.7)	0.122
VHDL-C, mmol/l	0.4 (0.3-0.6)		0.4 (35.3-0.6)	0.069
IA	2.9 (2.2-3.8)		2.7 (35.2-3.8)	0.014
Genotype				
	AA	AC	CC	
Age, years	47.0 (35.0-55.0)	48.0 (37.8-55.0)	33.0 (29.5-58.5)	0.576
WC, cm	88.5 (78.3-98.0)	86.0 (37.0-98.0)	81.0 (75.3-82.0)	0.032
SBP, mmHg	130.0 (120.0-150.0)	130.0 (37.0-150.0)	120.0 (117.5-145.0)	0.565
DBP, mmHg	80.0 (80.0-90.0)	80.0 (37.0-90.0)	80.0 (77.5-90.0)	0.569
FPG, mmol/l	4.5 (4.0-5.0)	4.3 (37.8-5.0)	3.8 (3.3-4.4)	0.121
TG, mmol/l	1.0 (0.7-1.4)	0.9 (37.7-1.4)	0.9 (0.7-1.1)	0.295
TC, mmol/l	4.9 (4.4-5.5)	4.9 (37.3-5.5)	4.9 (4.1-5.5)	0.669
HDL-C, mmol/l	1.2 (1.0-1.5)	1.3 (37.1-1.5)	1.4 (1.2-1.6)	0.027
LDL-C, mmol/l	3.2 (2.7-3.7)	3.0 (37.6-3.7)	3.1 (2.6-3.6)	0.261
VHDL-C, mmol/l	0.4 (0.3-0.7)	0.4 (37.3-0.7)	0.4 (0.3-0.5)	0.170
IA	3.0 (2.2-4.0)	2.7 (37.0-4.0)	2.6 (2.0-3.5)	0.034
	AA		AC+CC	
Age, years	47.0 (35.0-55.0)		47.5 (36.8-55.0)	0.750
WC, cm	88.5 (78.3-98.0)		84.5 (77.0-98.0)	0.127
SBP, mmHg	130.0 (120.0-150.0)		130.0 (120.0-150.0)	0.405
DBP, mmHg	80.0 (80.0-90.0)		80.0 (80.0-90.0)	0.292
FPG, mmol/l	4.5 (4.0-5.0)		4.2 (3.8-5.0)	0.194
TG, mmol/l	1.0 (0.7-1.4)		0.9 (0.7-1.4)	0.134
TC, mmol/l	4.9 (4.4-5.5)		4.9 (4.2-5.5)	0.394
HDL-C, mmol/l	1.2 (1.0-1.5)		1.3 (1.1-1.5)	0.007
LDL-C, mmol/l	3.2 (2.7-3.7)		3.0 (2.6-3.7)	0.102
VHDL-C, mmol/l	0.4 (0.3-0.7)		0.4 (0.3-0.7)	0.065
IA	3.0 (2.2-4.0)		2.7 (2.0-4.0)	0.010

Carriers of the A allele and AA genotype were characterized by a statistically significantly larger WC, lower HDL-C level, and high values of the atherogenic index (Table 3). In carriers of the AC+CC genotypes, the identified features persisted.

We found a significantly higher incidence of decreased HDL-C level in carriers of the A allele than in carriers of the C allele (38.1% vs. 27.7%, P=0.026), and a higher incidence in AA carriers than in carriers of the AC and CC genotypes (40.5%, 28.6%, 22.2%, respectively; P=0.005).

The HDL-C level did not correlate with the age of the subjects (r=-0.07, P=0.076). No correlation was found between the HDL-C level and SBP (r=-0.06, P=0.113), DBP (r=-0.09, P=0.013), FPG (r=-0.07, P=0.069), LDL-C (r=-0.03, P=0.412). Negative correlations were found between the HDL-C level and WC (r=-0.26, P<0.001) and TG levels (r=-0.58, P<0.001). The revealed differences in the HDL-C levels in carriers of different genotypes and alleles of the *AGTR1 A1166C* polymorphism were associated with differences in WC.

Conclusion

The obtained data show no association of the *AGTR1* A1166C polymorphism with EHT in the representatives of indigenous people of the Arctic territory of Yakutia. The limitations of the study were: the small number of groups and the inability to conduct a full, comprehensive examination of the participants to exclude the secondary nature of hypertension. A positive aspect of the research was the usage of controls from the same population, in the same time period. In further studies, an additional verification of the studied link would be possible by using the hospital population as "cases," excluding the secondary nature of hypertension

Competing Interests

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Population Genetics

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C3435T Polymorphism of the *ABCB1* gene in the Yakut Population

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Abstract

Background: The *ABCB1* gene is responsible for resistance to various cytotoxic drugs. The product of the *ABCB1* gene, P-glycoprotein (P-gp), acts as a transmembrane pump and influences the action of many drugs. More than 40 SNPs of the *ABCB1* gene that alter the expression of P-gp have been identified. The *ABCB1* rs1045642 SNP, designated as C3435T (C-the wild-type allele, T-the variant allele), correlates with the activity of P-gp. The aim of our research was to study the distribution of alleles and genotypes of the *ABCB1* C3435T polymorphism in Yakuts, in comparison with other human populations.

***Methods and Results*:** The studied cohort included 149 healthy Yakut volunteers (36 men and 113 women). The average age of participants was 30.67±0.06 years. The *ABCB1* gene is a highly polymorphic gene; the allele frequency of the C3435T polymorphism differs widely among the studied populations. The frequency of the mutant T-allele among the Yakuts was 51% .In the studied group of Yakuts, we revealed the prevalence of the heterozygous CT genotype (75.8%). The Yakuts have a relatively low frequency of CC (10.7%) and TT (13.4%) genotypes. This preliminary study did not include the objective of proving the relationship between the *ABCB1* C3435T polymorphism and addictive disorders in Yakuts. The further search for functional polymorphisms of the *ABCB1* gene and associations with addictive behavior using a systematic approach on larger samples is of great practical importance.**(International Journal of Biomedicine. 2021;11(3):367-371.)**

Key Words: ABCB1 • single nucleotide polymorphism • multidrug resistance • P-glycoprotein

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Abbreviations

AD, addictive disorders; **HWE**, Hardy-Weinberg equilibrium; **ABCB1**, ATP-Binding Cassette Subfamily B Member 1; **MDR**, multidrug resistance; **P-gp**, P-glycoprotein; **SNPs**, single nucleotide polymorphisms

Introduction

Since its first description by R. Juliano and V. Ling, the ATP-binding cassette (ABC) protein P-gp has become the object of special attention as a key player in one of the drug resistance mechanisms.(1) Multidrug resistance (MDR) is a biological phenomenon that significantly increases the survival of tumor cells under the treatment of cytostatic drugs, which ultimately negatively affects the life expectancy of patients with malignant neoplasms. The MDR phenotype is associated with the functioning of the products of the ABC-transporter family of genes, a function that is associated with a major drug resistance mechanism.(2)

P-glycoprotein (P-gp), a protein encoded by the *ABCB1* gene, is an important transporter for many drugs, and is also associated with many immunological processes and apoptosis. (3) The P-gp is a very broad-spectrum efflux pump that is present in a variety of endothelia: liver, jejunum, or brain

capillary endothelial cells. P-gp plays an important role in the blood-brain barrier, protecting neurons from xenobiotics.

The *ABCB* gene family includes the *ABCB1* and *ABCB2* genes. The *ABCB1* gene is responsible for resistance to various cytotoxic drugs; the exact functions of the *ABCB2* gene, however, remain unknown.[4] The product of the *ABCB1* gene, P-gp, acts as a transmembrane pump and influences the action of many drugs.[5] The *ABCB1* gene is localized on chromosome 7q21. More than 40 SNPs of the *ABCB1* gene that alter the expression of P-gp have been identified. Three SNPs (1236C>T, 2677G>T and 3435C>T) have been repeatedly shown to predict changes in P-gp function. It has been shown that the frequencies of these polymorphisms in a population are ethnically related.

The *ABCB1* rs1045642 SNP, designated as C3435T (C-the wild-type allele, T-the variant allele), correlates with the activity of P-gp. In individuals homozygous for the T-allele, the expression of P-gp is more than four times lower than in individuals with the CC genotype.

The relationship of P-glycoprotein with the resistance of cancer cells to chemotherapy has been identified. The polymorphisms in the *ABCB1* gene can affect the pharmacokinetics of many drugs, including cytostatic anticancer drugs.[3-5]

The expression and function of some xenobiotic carriers vary depending on the time of the day, causing time-dependent changes in the distribution and toxicity of the drug. P-gp is highly expressed in the kidneys and is involved in the excretion of various drugs. The elimination of several P-gp substrates was demonstrated to vary depending on administration time, but the underlying mechanism remains unclear.[6-8]

Lopez and co-authors found that *ABCB1* in the adrenal glands can regulate the adaptation to stress. They identified previously unknown subpopulation cells Abcb1b+ involved in stress adaptation in the adrenal glands. This finding was confirmed using a mouse stress model, adrenal tissues of patients with Cushing's syndrome, adrenal cell lines and peripheral cortisol, as well as genotyping data from patients with depression.[9]

There are also reports showing that the *ABCB1* gene participates in forming human resistance to various infections as a result of acquiring resistance to bacterial toxins or viruses.[10]

Muderrisoglu et al.[11] demonstrated that the genetic polymorphism of the *ABCB1(MDR1)* gene is significantly associated with non-smoking status in a cohort of Turkish people. In particular, the frequency of the *ABCB1* 1236TT-2677TT-3435TT haplotype was significantly higher in non-smokers than in smokers (21.5% vs. 10.8, respectively; *P*=0.018). This haplotype and associated allelic variants may serve as a biomarker of protection against nicotine addiction if confirmed by other studies in different populations.[11]

Another group of researchers suggest that the rs1045642 SNP has an adaptive significance, or is linked to other polymorphic sites that have an adaptive significance.[10]

In 2013, Isaza and colleagues[12] determined the prevalence of some genetic markers involved in addictive behavior in a group of addicts to derivatives of coca (cocaine/crack) or heroin and a control group of non-addicted people matched for gender, age, and ethnicity. Significant differences were found between drug addicts and the control group in relation to SNP 3435C>T of the *ABCB1* gene (*P*=0.001). The results showed that the 3435CC genotype is associated with addiction to heroin or cocaine.

Identification of these genetic markers will allow early identification of people at risk in order to take appropriate preventive measures and offer better treatment options for those suffering from AD.

The aim of our research was to study the distribution of alleles and genotypes of the *ABCB1* C3435T polymorphism in Yakuts, in comparison with other human populations.

Materials and Methods

The study of the *ABCB1* C3435T polymorphism was carried out in the Department of Molecular Genetics at YSC CMP. The studied cohort included 149 healthy Yakut volunteers (36 men and 113 women). The average age of participants was 30.67±0.06 years. All study participants filled out a questionnaire approved by the local committee on biomedical ethics at YSC CMP and voluntarily signed an informed consent to conduct genetic research.

Genomic DNA was isolated from the whole blood samples using a commercial DNA extraction and purification kit (Newteryx, Russia). All DNA samples were deposited to YSC CMP biomaterial collection "The Genome of Yakutia" (No. USU_507512).

Allelic variants of the *ABCB1* rs1045642 SNP were tested by PCR-RFLP assay. PCR conditions, sequences of oligonucleotide primers, the restriction enzymes used and lengths of restriction fragments are shown in Table 1.

Table 1.

Conditions of PCR-RFLP assay

Gene	Primer	AL, bp	AT, °C	RE	RFL, bp
ABCB1 (rs1045642)	F:5′-TTG ATG GCA AAG AAA TAA AGC-3′	207	54	DpnI	CC – 130,76 CT – 206,130,76 TT – 206
	R:5′-CTT ACA TTA GGC AGT GAC TCG-3′				

AL - amplicon length; AT - annealing temperature; RE - restriction enzyme; RFL - restriction fragment length

Genotypes were identified by analyzing the size of the resulting fragments by 4% agarose gel electrophoresis with ethidium bromide using a standard tris-acetate buffer at 120V for 1 hour. Restriction products were visualized using the Bio-Rad gel imaging system.

The study was approved by the Ethics Committee of the Yakut Science Center of Complex Medical Problems (YSC CMP). Written informed consent was obtained from each research participant (or the participant's parent/guardian).

Statistical analysis was performed using the Statistica 8.0 software package (Stat-Soft Inc., USA). The correspondence

of the distributions of genotypes to the expected values at HWE and comparison of the frequencies of allelic variants/genotypes were performed using the chi-square test. A probability value of $P<0.05$ was considered statistically significant.

Results

The *ABCB1* gene is a highly polymorphic gene; the allele frequency of the C3435T polymorphism differs widely among the studied populations. In terms of the allele frequency, the Yakuts are similar to the southern Kyrgyz, Russians, and Tuvans, and when compared to the data of other researchers and the open-source data of the 1000 Genomes Project, the frequency is observed to be similar to that of Caucasian populations (Table 2).

The greatest difference in the allele frequency between the studied group and other ethnic groups was observed with Africans, Peruvians, and Chinese (C allele: 60-88%). The highest frequency of the wild C allele was observed in Esan in Nigeria (88%). The frequency of the mutant T-allele among the Yakuts was 51%, which is comparable with previously published data on the southern Kyrgyz (51%),(10) as well as with the 1000 Genome Project data on the Punjabi Pakistanis (51%). The highest frequency of the T-allele (61%) was observed in Bengalis and northern Kyrgyz. The lowest frequency of the T-allele (12%-19%) was observed in African populations. We assume that the presence of the T-allele, as a result of acquiring resistance to bacterial toxins and viruses, helped to establish resistance to various diseases; at the same time, the presence of the C allele favored the assimilation

Table 2.

The frequency distribution of alleles and genotypes in of the ABCB1 C3435T polymorphism in different ethnic populations

Populations	n	Allele frequency		Genotype frequency			H_o	H_e	Fis	References
		C	T	CC	TC	TT				
Yakuts (Russia)	149	0.49	0.51	10.7	75.8	13.4	0.76	0.50	-0.52	Current study
Russians (Russia)	90	0.43	0.57	-	-	-	0.44	0.49	0.10	[10]
Tuvans (Russia)	142	0.43	0.57	-	-	-	0.46	0.49	0.06	
Northern Kyrgyz (Kyrgyzstan)	41	0.39	0.61	-	-	-	0.60	0.47	-0.28	
Southern Kyrgyz (Kyrgyzstan)	44	0.49	0.51	-	-	-	0.42	0.50	0.16	
Jordanians (Jordan)	337	0.57	0.43	33.2	47.5	19.3	0.47	0.49	0.04	[21]
Brazilians (Brazil)	278	0.55	0.45	23.7	61.5	14.7	0.61	0.50	-0.22	[22]
Maharashtrians (India)	222	0.42	0.58	18.0	47.3	34.7	0.47	0.49	0.04	[23]
Macedonians (Macedonia)	107	0.51	0.49	25.2	52.3	22.4	0.52	0.50	-0.04	[24]
Ashkenazi Jews (USA)	101	0.50	0.50	30.7	38.6	30.7	0.36	0.50	0.28	[25]
African in Caribbean in Barbados	96	0.85	0.15	71.9	26.0	2.1	0.26	0.26	0.00	[26]
Descendants of Africans from the Southeastern States (USA)	61	0.81	0.19	67.2	27.9	4.9	0.28	0.31	0.10	
Esan (Nigeria)	99	0.88	0.12	77.8	21.2	1.0	0.21	0.20	-0.05	
Gambians (Gambia)	113	0.81	0.19	66.4	29.2	4.4	0.29	0.31	0.06	
Luhya (Kenya)	99	0.86	0.14	72.7	26.3	1.0	0.26	0.24	-0.08	
Mende (Sierra Leon)	85	0.85	0.15	70.6	29.4	0.0	0.70	0.46	-0.52	
Yoruba (Nigeria)	108	0.87	0.13	77.8	19.4	2.8	0.19	0.22	0.14	
Colombians (Colombia)	94	0.44	0.56	31.9	47.9	20.2	0.48	0.49	0.02	
Mexicans (Mexico)	64	0.52	0.48	25.0	54.7	20.3	0.55	0.50	-0.10	
Peruvians (Peru)	85	0.62	0.38	36.5	51.8	11.8	0.52	0.47	-0.11	
Puerto Ricans (Puerto Rico)	104	0.57	0.43	35.6	43.3	21.2	0.43	0.49	0.12	
Daians (China)	93	0.57	0.43	34.4	46.2	19.4	0.46	0.49	0.06	
Han (Beijing)	103	0.62	0.38	41.7	40.8	17.5	0.41	0.47	0.13	
Han (South China)	105	0.69	0.31	50.5	38.1	11.4	0.38	0.42	0.10	
Japanese (Japan)	104	0.52	0.48	24.0	55.8	20.2	0.56	0.50	-0.12	
Kinh (Vietnam)	99	0.60	0.40	37.4	44.4	18.2	0.44	0.48	0.08	
Utahs of Northern and Western European Descent (USA)	99	0.43	0.57	18.2	50.5	31.3	0.51	0.49	-0.04	
Finns (Finland)	99	0.42	0.58	14.1	56.6	29.3	0.57	0.49	-0.16	
British (England and Scotland)	91	0.47	0.53	22	50.5	27.5	0.51	0.50	-0.02	
Iberians (Spain)	107	0.54	0.46	29.9	47.7	22.4	0.48	0.50	0.04	
Tuscan (Italy)	107	0.53	0.47	29.9	46.7	23.4	0.47	0.50	0.06	
Bengalis (Bangladesh)	86	0.39	0.61	14	50	36	0.50	0.48	-0.04	
Gujaratis living in Houston, exas (USA)	103	0.43	0.57	19.4	47.6	33	0.48	0.49	0.02	
Telugu (UK)	102	0.41	0.59	19.6	42.2	38.2	0.42	0.48	0.13	
Punjabi (Pakistan)	96	0.49	0.51	22.9	52.1	25	0.52	0.50	-0.04	
Tamils Sri Lanka (UK)	102	0.41	0.59	19.6	42.2	38.2	0.42	0.48	0.13	

H_o - observed heterozygosity, H_e - expected heterozygosity, Fis - Wright's fixation index.

of various drugs. However, this hypothesis needs further investigation.

The SNP genotyping of the *ABCB1* gene in the studied group of Yakuts revealed the prevalence of the heterozygous CT genotype (75.8%). Among all compared ethnic groups, the Yakuts had the highest heterozygosity. The level of genetic differentiation of populations in terms of frequencies, calculated using the F_{is} coefficient, was -0.52. Possibly the high heterozygosity of this polymorphism is associated with adaptation since carriers of both alleles experience their effects simultaneously. The Yakuts have a relatively low frequency of CC (10.7%) and TT (13.4%) genotypes. The lowest heterozygosity was observed in Yoruba (19.4%). A high frequency of the CC genotype was observed in African populations (66.4%-77.8%). Among Asian populations, a high frequency of the CC genotype was found in the Chinese (41.7%-50.5%).

The *ABCB1* C3435T polymorphism is a silent mutation, albeit with functional implications. Hoffmeyer observed a significant correlation of a polymorphism in exon 26 (C3435T) of *MDR-1* with expression levels and function of *MDR-1*. TT homozygous for this polymorphism showed significantly lower duodenal P-gp expression, higher in vivo activity of Pgp and increased digoxin plasma levels.[13] In a study by Wang et al.,[14] it was found that the abundant 3435C>T SNP appears to be a main factor in allelic variation of *ABCB1* mRNA expression in the liver, by changing mRNA stability.

A recent population pharmacokinetic study investigated the functional effects of common combinations of SNPs (C1236T, G2677T/A, and C3435T) and haplotypes of the *ABCB1* gene in vivo, measuring the apparent bioavailability of digoxin, which is a substrate of *ABCB1*. Carriers of CGC/CGT and TTT/TTT had 35% higher apparent bioavailability compared to the reference group CGC/CGC, while no difference was seen in CGC/TTT carriers.[15] The obtained results support the use of digoxin as a phenotyping substrate of intestinal P-gp activity. These results are also consistent with another report, showing about twice the frequency of the all-variant TT-TT-TT haplotype in non-smokers, since *ABCB1* is involved in the transport of endogenous compounds such as opioid peptides, steroids, glutamate and endorphin, which act as modulators of neurons in the central nervous system and may play a role in substance addiction mechanisms.[11]

It has been reported that the *ABCB1* polymorphism has an effect on substance abuse. For example, the *ABCB1* polymorphism affects methadone dosage in the treatment of opioid or heroin addiction.[16,17] In addition, nicotine has been shown to alter the expression of *ABCB1*[18,19] and long-term exposure to the carcinogen 4-methylnitrosamino-1-3-pyridyl-1-butanone (NNK) found in tobacco smoke plays a role in head and neck squamous cell carcinoma by increasing anti-apoptosis and therapeutic resistance via the Snail-RKIP signaling pathway.[20]

Therefore, it can be assumed that genetic polymorphisms of the *ABCB1* gene have an effect on the mechanisms associated with substance addiction, including nicotine addiction. However, the association of molecular and biological mechanisms has not been investigated. Our study was limited due to the inability to study the C1236T(rs1128503) and G2677T/A(rs2032582) polymorphisms for haplotype analysis and due to a relatively small cohort of studied people.

In conclusion, the comparative analysis of our results with literature data on other human populations showed a high observed heterozygosity (75.8%) in the Yakut population. The frequencies of the CC and TT genotypes in the studied group were relatively low: 10.7% and 13.4%, respectively. This preliminary study did not include the objective of proving the relationship between the *ABCB1* C3435T polymorphism and addictive disorders in Yakuts. The further search for functional polymorphisms of the *ABCB1* gene and associations with addictive behavior using a systematic approach on larger samples is of great practical importance.

Competing Interests

The authors declare that they have no competing interests.

Sources of Funding

The study was carried out within the framework of the project “The study of the genetic structure and burden of hereditary pathology of populations of the Republic of Sakha (Yakutia).” All DNA samples were deposited to YSC CMP biomaterial collection “The Genome of Yakutia” (No. USU_507512).

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ORIGINAL ARTICLE

Experimental Biology

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Combined Correction of Experimental Critical Ischemia of the Lower Extremities in a Rat Model

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Abstract

The purpose of our research was to study the effectiveness of correcting experimental critical ischemia (CI) of the lower extremities with a combination of udenafil, simvastatin, and autologous bone marrow mononuclear cells (ABMMC).

Methods and Results: The experiments were carried out on 24-month-old Wistar rats, weighing 220–250g. The animals were randomized by sex and weight. Groups were formed according to the manipulations carried out during the operations. The animals were divided into 7 groups, each with 20 animals: Group 1 included intact animals; Group 2 - falsely operated animals; Group 3 (control group) - animals with simulated CI without treatment; Group 4- animals with CI and monotherapy with udenafil (daily oral administration of 8.6 mg/kg for 28 days); Group 5 - animals with CI and simvastatin monotherapy (daily oral administration of 1.71 mg/kg for 28 days); Group 6 - animals with CI and monotherapy with ABMMC (parenterally, once on Day 7 after modeling CI, 50 µl at 4 points and, paravasally, above the inguinal ligament in the area where the lateral artery leaves the artery enveloping the femur from the internal iliac artery; in the area of the superficial artery that bends around the iliac bone under the inguinal ligament; into the area of origin of the muscular branch of the femoral artery r. muscularis, the place of attachment of the comb and long adductor muscles of the thigh; in the upper third of the gastrocnemius muscle]); Group 7 - animals with CI and combination therapy (udenafil and simvastatin drugs were administered intragastrically 0.86 mg/kg, once a day, for 7 days) and one-time parenteral administration of ABMMC, according to the same scheme as in Group 6. On Days 21 and 28 of the experiment, the level of blood microcirculation was determined in the muscles of the rat leg; for this, laser Doppler flowmetry was used. For further morphometric assessment of the leg muscles, they were removed. Preparations for morphometric analysis were prepared according to the standard technique with Van Gieson staining, as well as H&E. Our study demonstrated the effectiveness of combination therapy with udenafil, simvastatin, and ABMMC to correct critical lower limb ischemia in rats. The severity of morphological changes on the background of this combination was minimal, compared to the findings of other study groups, and the level of blood microcirculation in the ischemic zone on Day 28 was, significantly, 1.9 times higher than in animals of the control group.

Conclusion: The results obtained allow us to recommend the use of the investigated combination (udenafil+simvastatin+ABMMC) for the treatment of patients with critical limb ischemia, both in outpatient and inpatient practice. **(International Journal of Biomedicine. 2021;11(3):372-375.)**

Key Words: critical ischemia • microcirculation • autologous bone marrow mononuclear cells

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Abbreviations

ABMMC, autologous bone marrow mononuclear cells; **CG**, control group; **CI**, critical ischemia; **EG**, experimental group; **H&E**, hematoxylin and eosin; **PU**, perfusion units.

Introduction

Because critical ischemia (CI) of the lower extremities leads to disability and death not only in the elderly, but also in people of working age, it is one of the serious diseases of the cardiovascular system. CI requires a timely and accurate assessment of the patient's condition, analysis of the dynamics of the development of this pathology, as well as intensive treatment.[1,2] Most often, surgical methods of treatment are used to treat this pathology; however, there are 20%-25% of patients with diffuse vascular lesions and without a distal vascular bed, for whom the use of this method of treatment is limited.[3,4] In the last decade, phosphodiesterase-5 inhibitors, which were originally used as a means of correcting erectile dysfunction, have been actively studied. They are currently being investigated for further use in cardiology, gynecology, gastroenterology and many other related medical specialties. Phosphodiesterase-5 inhibitors have vasodilating activity and are effective in correcting skeletal muscle ischemia.[5,6] To correct CI of the lower extremities, the use of drugs of this group, in our opinion, is pharmacoeconomically more expedient than drugs of the group of synthetic prostaglandins. Statins are of particular interest for the prevention and treatment of atherosclerosis and coronary artery disease. In addition to their main activities, the drugs of this group have many pleiotropic effects: improved endothelial function; anti-inflammatory, antiplatelet, antioxidant effects; slowing down the proliferation of smooth muscle cells in the vascular wall; and stimulation of fibrinolysis, all of which do not depend on hypocholesterolemic activity in drugs of this group. These drugs also have an inhibitory effect on the synthesis of pro-inflammatory cytokines:[7] interleukins-(1,6,8) and TNF-α. In addition, in patients with hypertriglyceridemia, statins normalize the concentration of triglycerides.[8] There are also studies that have demonstrated experimentally the effectiveness of ABMMC for the treatment of critical limb ischemia. This treatment is effective because ABMMC contains stem cells, such as hematopoietic and mesenchymal. These cells contribute to the replacement of tissue defects and neoangiogenesis since they have multipotent properties.[9] Thus, the use of a combination of udenafil, simvastatin and ABMMC in the case of limb ischemia, acting on different links in the pathogenesis of this pathology, may be completely justified.

The purpose of our research was to study the effectiveness of correcting experimental CI of the lower extremities with a combination of udenafil, simvastatin, and ABMMC.

Materials and Methods

The experiments were carried out on 24-month-old Wistar rats, weighing 220–250g. Before the study, the experimental animals were quarantined for 7 days in the vivarium of the Research Institute of EM at the Kursk State Medical University. The rats were kept in a standard room with 12 hours of automatic lighting and air temperature maintained at 22–24 °C.

The animals were randomized by sex and weight. Groups were formed according to the manipulations carried out during the operations. The animals were divided into 7 groups, each with 20 animals: Group 1 included intact animals; Group 2 - falsely operated animals; Group 3 (CG) - animals with simulated CI without treatment; Group 4 (EG1) - animals with CI and monotherapy with udenafil; Group 5 (EG2) - animals with CI and simvastatin monotherapy; Group 6 (EG3) - animals with CI and monotherapy with ABMMC (parenterally, once on Day 7 after modeling CI, 50μl at 4 points and, paravasally, above the inguinal ligament in the area where the lateral artery leaves the artery enveloping the femur from the internal iliac artery; in the area of the superficial artery that bends around the iliac bone under the inguinal ligament; into the area of origin of the muscular branch of the femoral artery *r. muscularis,* the place of attachment of the comb and long adductor muscles of the thigh; in the upper third of the gastrocnemius muscle]); Group 7 (EG4) - animals with CI and combination therapy (intragastrically udenafil and simvastatin) and one-time parenteral administration of ABMMC, according to the same scheme as in Group 6.

All experimental manipulations were performed in the afternoon. The animals were anesthetized with chloral hydrate, introduced intraperitoneally at a dose of 300 mg/kg in an aqueous solution that was prepared in advance on the day of the experiment. Before surgery, the rats were fixed on a special table, after which the hair was shaved off on the inner surface of the thigh and lower leg, the incision area was treated with a 70% aqueous solution of alcohol. The rats were removed from the experiment by means of an overdose of anesthesia. In the group of sham-operated rats, after anesthesia, all experimental animals were operated on through the skin along the surface of the thigh and lower leg from the inside, and the neurovascular bundle was isolated, after which the wound was sutured. The CG was simulated similarly to the sham-operated rats, but with the removal of a portion of the great vessels, such as the femoral, popliteal, anterior, and posterior tibial arteries and veins, as well as the sciatic nerve.[10] In Group 4, the experimental pathology was corrected daily, for 28 days, by oral administration of udenafil 8.6 mg/kg, and in Group 5 - daily, for 28 days, with oral administration of simvastatin 1.71 mg/kg. In Group 6, CI was corrected by introducing ABMMC (from the tibia by puncture of the bone marrow cavity and cell isolation according to the Boyum method), a single parenteral injection on Day 7 after modeling CI, 50μl at 4 points, as described above. In Group 7, CI was corrected with a combination of udenafil and simvastatin (both drugs were administered intragastrically 0.86 mg/kg, once a day, for 7 days) and a single ABMMC injection, similar to Group 6.

On Days 21 and 28 of the experiment, the level of blood microcirculation was determined in the muscles of the rat leg; for this, laser Doppler flowmetry was used. In our study, we used the TSD144 needle sensor included in the kit for the LDF100C hardware complex. The data were recorded and analyzed using the AcqKnowledge 3.8.1 program included in the BIOPAC Systems. Blood microcirculation indices were measured in perfusion units (PU). After that, for further morphometric assessment of the leg muscles, they were removed. All biomaterial for histology was fixed in 10% formalin aqueous solution for 7 days. Preparations for morphometric analysis were prepared according to the

standard technique with Van Gieson staining, as well as H&E. A Levenhuk 320 microscope (USA) with a Levenhuk C310 digital camera was used for morphometric analysis. The processing was carried out using the ScopeTek ScopePhoto 3.1.268 program. Considering the data of previous studies, the most informative were the results obtained on Day 28. We considered the results that were obtained on Day 21 as additional.

In vivo experiments were carried out in accordance with the legislation of the Russian Federation, in strict compliance with the European Convention for the protection of animals used for experimental and other purposes (Strasbourg, France, 1986), the provisions of Directive 210/63/EU of the European Parliament and the Council of the European Union of 22 September 2010 on the protection of animals used for scientific purposes (Article 27), and approved by the Regional Ethics Committee of Kursk State Medical University (Protocol No. 4 dated June 10, 2019).

Statistical analysis was performed using the STATISTICA software package (v.12.0, Stat-Soft Inc., USA). The mean (M) and standard error of the mean (SEM) were calculated. Student's unpaired and paired t-tests were used to compare two groups for data with normal distribution. Differences of continuous variables departing from the normal distribution, even after transformation, were tested by the Mann-Whitney U-test. The Wilcoxon criterion was used to compare the differences between the paired samples. A value of $P<0.05$ was considered significant.

Results

In the leg muscles of intact rats on Days 21 and 28 of the experiment, the average microcirculation level was 531.6±12.1 PU. Microscopic analysis of histological sections of the region of the middle third of the tibia revealed well-defined longitudinally and transversely cut symplasts of skeletal muscle tissue, connective tissue layers forming endo-, peri- and epimysium of muscle fibers, and small and large blood vessels. Blood vessels had no pathological changes. In the sarcoplasm of the muscles, transverse striation was clearly visualized, as well as elongated basophilic nuclei of a dark shade (Fig. 1A).

Sham-operated rats showed no significant differences, compared to intact rats, in the structure of muscle tissue and indicators of the level of microcirculation (523±13.8 PU on Day 21 ($P=0.645$) and 524.1±15.1 PU on Day 28 ($P=0.625$)) (Fig.1B). However, the rats of the CG showed significant differences, compared to intact rats (249.1±7.3 PU on Day 21 ($P<0.05$) and 302.9±6.5 PU on Day 28 ($P<0.05$). On Day 21, a high degree of tissue reactivity was observed in the affected muscles. Signs of inflammation were pronounced. In the immediate vicinity of the areas of necrosis, bundles of atrophied muscle fibers were periodically visualized. In the layers between the symplasts of the skeletal muscle, the cellular component prevailed over the fibrous one. Primarily lymphocytes, monocytes, eosinophils, single fibroblasts, and fibrocytes were visualized in the field of view. In the epimysium, tissue surrounding the muscle, there were signs of interstitial edema as well as newly formed blood-filled capillaries with signs of perivascular round-cell infiltration. On Day 28 of the experiment, there was a decrease in reactivity tissues with partial normalization of muscle color; however, they were somewhat hypotrophic. The necrotic areas were somewhat smaller. It should be noted that full-blooded, single capillaries were formed (Fig.1C).

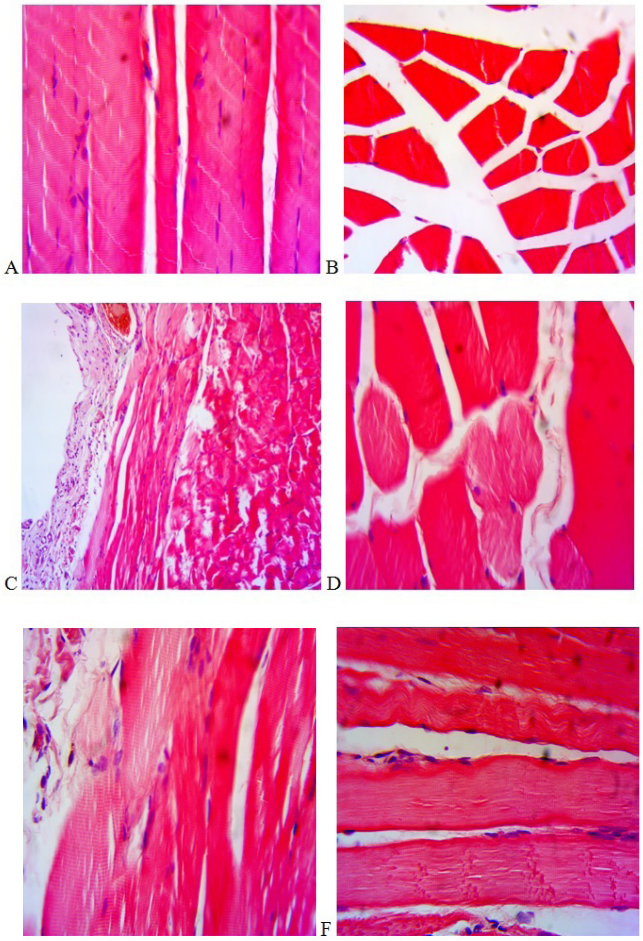

Fig. 1. *Micrograph of a section of skeletal muscle tissue in the region of the middle third of the rat leg on Day 28 of the experiment. H&E staining (×400). A - muscles of the leg of the intact rats; B - muscles of the lower leg of the rats of the sham-operated group; C - ischemic muscles of the lower leg of the rats of the CG; D - ischemic leg muscles of rats treated with udenafil; D - ischemic leg muscles of rats treated with simvastatin; E - ischemic muscles of the leg of rats after administration of ABMMC; F - ischemic leg muscles of rats treated with udenafil, simvastatin in combination with ABMMC.*

In Group 4 with udenafil monotherapy, the level of regional blood flow was significantly higher than in the CG at the corresponding time and was 394.6±6 PU on Day 21 ($P<0.05$) and 486±7.6 PU on Day 28 ($P<0.05$). The level of microcirculation in this group on Days 21 and 28 approached the values of the group of intact animals. Microscopy revealed small foci of atrophying myocytes with cell proliferation, and a decrease in the size and number of necrotic areas due to the formation of young connective tissue. However, there was round-cell infiltration of connective tissue layers between skeletal muscles, small blood vessels (arterioles and venules) were dilated with signs of

thrombosis, and a new capillary network was formed near the sites of necrosis (Fig.1D). In Group 5, simvastatin monotherapy also had a positive effect on microcirculation indices (382.6±4.3 PU on Day 21 (P<0.05) and 468.5±6.8 PU on Day 28 (P<0.05). The histological picture was similar to the EG1, but the expansion of small blood vessels was less pronounced (Fig.1E). In Group 6 with ABMMC monotherapy, the level of regional blood flow was 401.3±10.9 PU on Day 21 (P<0.05) and 504.8±8.5 PU on Day 28 (P<0.05). As in the EG1 group, microscopy revealed a decrease in the number and size of necrotic areas. Circular cell infiltration of connective tissue layers between skeletal muscles continued, small blood vessels were dilated with signs of thrombosis, and a new capillary network was formed near the sites of necrosis. There were local areas with insufficiently stained sarcoplasm and no transverse striation. The density of cells was high, cells of the fibroblastic series predominated in the field of view, and single lymphocytes were visualized. In the interlayers between the symplasts of hypertrophied mast cells, which were in the stage of secretion accumulation and degranulation (Fig.1E). In the EG4, the level of regional blood flow was 493.4±17.5PU on Day 21 (P<0.05) and 574.7±9.9 PU on Day 28 (P<0.05). The severity of morphological changes in this group was minimal among all observation groups. Symplasts of muscle fibers have a uniform sarcoplasmic coloration. When imaging at high magnification, both on transverse and longitudinal sections, small elongated nuclei located along the periphery of the muscle fibers were visualized. In the layers of connective tissue, the density of cells was low; in the field of view of the fibroblast prevailed. Mast cells were small, mainly in the stage of secretion accumulation, and lymphocytes were single. Small blood vessels were filled with blood, without signs of structural abnormalities. A large number of newly formed blood vessels with signs of thrombosis were visualized in the wide layers of connective tissue (in the areas between the muscles where large vessels were originally localized). We found large blood vessels without significant structural changes. Endotheliocytes were flat, the cell axis was oriented along the basement membrane, and the muscular membrane was formed by smooth myocytes, without signs of fibrosis (Fig.1F).

In conclusion, our study demonstrated the effectiveness of combination therapy with udenafil, simvastatin, and ABMMC to correct critical lower limb ischemia in rats. The severity of morphological changes on the background of this combination was minimal, compared to the findings of other study groups, and the level of blood microcirculation in the ischemic zone on Day 28 was, significantly, 1.9 times higher than in animals of the CG. The results obtained allow us to recommend the use of the investigated combination (udenafil+simvastatin+ABMMC) for the treatment of patients with critical limb ischemia, both in outpatient and inpatient practice.

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Competing Interests

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Experimental Biology

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Morphofunctional Rearrangement of the Fibrous Structures of the Rat Dermis under the Conditions of Implantation of 3D Scaffold Based on Polyprolactone

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Abstract

Background: the use of various scaffolds allows us to model the future fibrous framework of the newly formed regenerate, and also serves as a substrate for the settlement of the cellular component. The development of tissue engineering in regenerative medicine demands an understanding of the more specific mechanisms of the formation of the connective framework at the site of the defect. The aim of this research was to study the morphofunctional rearrangement of the fibrous structures of the rat dermis in response to the implantation of a 3D scaffold based on polyprolactone.

***Methods and Results*:** The experiment was performed on 30 white male Wistar rats. The object of the study was a skin fragment together with an implantable 3D scaffold based on polyprolactone, taken on Days 3, 7 and 14 after implantation. Biomaterial with implantable scaffold was studied using light and scanning electron microscopy. The results of the study indicate that the 3D scaffold based on polyprolactone has good biocompatibility, causing a weak inflammatory reaction, and contributes to the formation of the connective tissue framework by Day 14.

Conclusion: The results of the study can be used to develop new scaffolds or modify existing ones, as a "framework" for populating the cellular component and creating tissue-engineering structures.(**International Journal of Biomedicine. 2021;11(3):376-378.**)

Key Words: skin • 3D scaffold • regeneration • collagen fibers

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Introduction

Recently, a large number of studies have appeared on the development of scaffolds for the replacement of skin defects. This is primarily due to the need to improve the effectiveness of treatment of burns and chronic wounds, as well as re-epithelization of large areas of damaged skin during surgical operations. The created dermal scaffolds should contribute to the improvement of healing indicators (reduction of the inflammatory response, formation of granulation tissue, stimulation of angiogenesis, acceleration of wound epithelization, etc.) and reduction of complications.[1,2] Currently, there is no doubt that the effectiveness of artificial extracellular matrices in stimulating tissue regeneration is associated with providing sufficient temporary mechanical support for the formation of a new fibrous backbone.[3-5]

To assess the prospects of scaffolds, morphological studies of tissue response to the implantation of scaffolds in vivo and the study of the features of the formation of the collagen framework are necessary.[6,7] Such studies are not numerous in the scientific literature. Based on the above, the aim of our research was to study the morphofunctional rearrangement of the fibrous structures of the rat dermis

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in response to the implantation of a 3D scaffold based on polyprolactone

Materials and Methods

The experiment was performed on 30 white male Wistar rats, weighing 150±15 g. The object of the study was a skin fragment together with an implantable 3D scaffold based on polyprolactone, taken on Days 3, 7 and 14 after implantation. The biomaterial was taken from the lateral surface of the back, by excision of the skin of the specified size, to the fascia of the subcutaneous muscle.

In vivo experiments were carried out in accordance with the legislation of the Russian Federation, in strict compliance with the European Convention for the protection of animals used for experimental and other purposes (Strasbourg, France, 1986), the provisions of Directive 210/63/EU of the European Parliament and the Council of the European Union of 22 September 2010 on the protection of animals used for scientific purposes (Article 27), and approved by the Regional Ethics Committee of Kursk State Medical University (Protocol No. 4 dated June 10, 2019).

For light microscopy, the material was fixed in a 10% aqueous solution of neutral formalin. Microtomization and filling in with paraffin were carried out according to standard prescriptions. Sections with a thickness of 5-7 microns were stained by H&E. For scanning electron microscopy (SEM), the skin was fixed with 10% buffered neutral formalin, dehydrated in a frozen state in alcohols of increasing concentrations. The samples prepared in this way were mounted on a special aluminum table with a conductive carbon glue, sprayed with gold or platinum-paladium alloy in a Quorum Q150TS spray unit (GaLa Gabler Labor Instrumente. Handels GmbH, Germany) and viewed in an SEM S 3400N (Hitachi, Japan).. Next, the scanned image was processed using the ImageJ program, where the thickness of the collagen fibers surrounding the threads of the polyprolactone matrix was measured in each case in 50 fields of view.

Statistical analysis was performed using the STATISTICA software package (v.12.0, Stat-Soft Inc., USA). For descriptive analysis, results are presented as median (Me), first quartile (*25th percentile*) and third quartile (*75th percentile*). 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

On Day 3 of the experiment, the fibrous structures were disorganized in the dermis. At the same time, on the cross sections, their shape varied from flattened to rounded. Large inter-fibrous gaps were determined between the fibers, which may indirectly indicate the preservation of interstitial edema by this time. Also, in the field of view, were a large number of individual fibrils, as well as some combined into fibers (Figure 1).

By Day 7, we observed active cell migration at the implantation site. The cellular composition is mainly represented by lymphocytes, numerous macrophages and single fibroblastic cells. When studying the fibrous component around the scaffold threads, we found collagen fibers strictly oriented in one direction, the thickness of which was 0.13[0.09;0.16] microns. The orientation of the collagen structures followed the shape of the implant structures – they spread both circularly around the scaffold threads, and in parallel, sprouting between the polyprolactone threads (Figure 2).

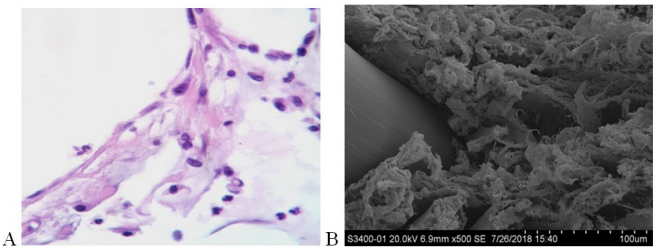

Fig. 1. *Microphotography of the rat skin dermis on the site of the implanted 3D scaffold based on polycaprolactone on Day 3 of the experiment. A - H&E staining (× 400). B - SEM (× 500)*

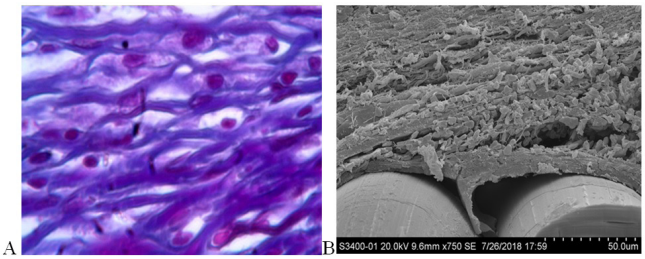

Fig. 2. *Microphotography of the rat skin dermis on the site of the implanted 3D scaffold based on polycaprolactone on Day 7 of the experiment. A - H&E staining (× 400). B - SEM (× 1000)*

On Day 14 of the experiment, an increase in the number of collagen structures was observed around the matrix structures. The fiber thickness increased to 0.24[0.18;0.84] microns. At the same time, a certain spatial pattern was noted – sagittal directed fibers were located more compactly, and had practically no inter-fiber gaps. The frontal fibers were more loosely arranged; they were thin and had a large number of branches (Figure 3).

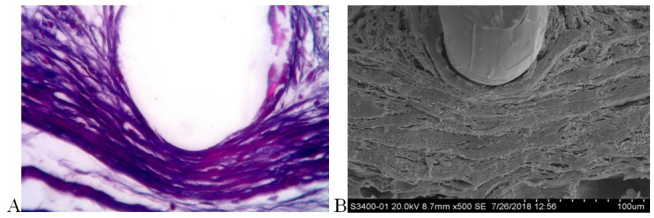

Fig. 3. *Microphotography of the rat skin dermis on the site of the implanted 3D scaffold based on polycaprolactone on Day 14 of the experiment. A - H&E staining (× 200). B - SEM (× 500)*

This organization of fibrous structures is the basis for the construction of the connective tissue capsule and the germination of blood vessels into it; in addition, it allows us to judge the completion of the stage of adaptation and

reconstruction of the dermis for the further formation of the connective tissue capsule that separates the scaffold from the surrounding tissue.

Tissue engineering is currently considered the leading field in regenerative medicine. Tissue-engineered structures consisting of scaffolds (matrices) and cells cultured on them are used in reconstructive operations in various fields of medicine. These properties, as well as the possibility of modeling a three-dimensional porous structure similar to natural extracellular matrices, allow us to create conditions for organotypic regeneration. The results of the study indicate that the 3D scaffold based on polyprolactone has good biocompatibility, causing a weak inflammatory reaction, and contributes to the formation of the connective tissue framework by Day 14.

Comparing our data with that of other authors, the optimal time for the beginning of resorption of such materials should be the period from 10 to 14 days, which is necessary for maintaining normal metabolism, proliferative activity and differentiation of cells and, as a result, for determining the possibility of vascularization and remodeling of regenerating tissue.[8-10]

In conclusion, our experimental study showed that the use of a 3D scaffold based on polyprolactone contributes to a faster formation of the collagen framework at the implantation site. The results of the study can be used to develop new scaffolds or modify existing ones, as a "framework" for populating the cellular component and creating tissue-engineering structures.

Competing Interests

The authors declare that they have no competing interests.

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