Situation modeling
9 Mechanisms of cancellation by pain of genetically determined inhibition of a malignant tumor growth in experiment
Oleg I. Kit, Elena M. Frantsiyants, Alla I. Shikhlyarova, Inga M. Kotieva, Irina V. Kaplieva
We used 48 C57BL/6-Plau6mI.1Bug-ThisPlau6FDhu/GFD-hu mice of both genders with urokinase gene knockout and 102 C57BL/6 mice of both genders with the normal genotype. Chronic neurogenic pain (CNP) was produced due to bilateral ligation of the sciatic nerve.

18 Relationship between comorbid pathology and tumor progression. Morphological portrayal of internal organs in modeling the growth of Guerin's carcinoma under diabetic conditions
Alla I. Shikhlyarova, Oleg I. Kit, Elena M. Frantsiyants,
Irina V. Kaplieva, Galina V. Zhukova,
Irina V. Neskubina, Lidia K. Treptitaki,
Yuriy V. Przhezdetskiy, Viktoria V. Pozdnyakova,
Yulia Yu. Kozel, Dmitry P. Atmachiid,
Alexandra A. Vereskunova, Stella M. Babieva,
Inga M. Kotieva, Maria I. Morazova
An increase in the incidence of malignant tumors progressing against the background of various comorbid pathologies determines the need to study the mutual influence of pathological processes using experimental modeling.

27 Influence of diabetes mellitus on free radical processes in the heart in rats with Guerin's carcinoma and characteristics of malignant lesions depending on the gender of animals
Oleg I. Kit, Elena M. Frantsiyants, Irina A. Goroshinskaya,
Irina V. Kaplieva, Alla I. Shikhlyarova,
Lyudmila A. Nemashkalova, Lidia K. Treptitaki,
Polina S. Kachesova, Oksana V. Bykadorova,
Elizaveta V. Serdyukova, Natalia A. Maksimova,
Maria G. Ichenko, Lydymila G. Akopian,
Elena I. Agarkova, Marina M. Sergeeva,
Inga M. Kotieva, Maria I. Morazova
To study the intensity of lipid peroxidation (LPO) and the activity of the main antioxidant protection enzyme: superoxide dismutase (SOD) in heart tissues and tumors in rats of both genders with Guerin's carcinoma (GC)...

34 Modifying effect of obesity on the content of sex hormones and their receptors in endometrial adenocarcinoma and its surrounding tissue
Oleg I. Kit, Elena M. Frantsiyants,
Valeria A. Bandovkina, Tatiana I. Moiseenko,
Natalia V. Chernikova, Meri L. Adamyan,
Yuriy A. Poryvaev, Natalia D. Cheryarina,
Sergey V. Tumanyan, Svetlana V. Kornienko
Obese EC patients showed longer healing of postoperative wounds, slow recovery, and more frequent tumor metastasizing to regional lymph nodes. In the tumor samples in all patients, compared with the intact endometrium, the levels of estrogens, testosterone and their receptors were higher.

41 Development of an experimental model of tumor growth under hypothyroidism
Oleg I. Kit, Elena M. Frantsiyants,
Valeria A. Bandovkina, Irina V. Kaplieva,
Yulia A. Pogorelova, Lidia K. Treptitaki,
Irina V. Neskubina, Alla I. Shikhlyarova,
Natalia D. Cheryarina, Polina S. Kachesova,
Lyudmila A. Nemashkalova, Ekaterina I. Surikova,
Irina A. Goroshinskaya, Galina V. Zhukova,
Victoria L. Volkova, Natalia A. Chertova,
Marina A. Engibaryan, Alina Y. Arakelova,
Aina M. Salatova
Our aim has been to develop an experimental model of the tumor growth against the background of hypothyroidism in rats of both genders in order to study possible influence made by hypothyroidism on progression of malignant tumors of various histological types.

50 Decision support system in radiology for fast diagnostics of thoracic diseases under COVID-19 pandemic conditions
Ilya M. Borodyansky
In the present article the relevance of using DSS under the current conditions for image recognition and, as a more specific application, for the purpose of additional assistance rendered to medical experts (radiologists) in their decision-making and preparing findings upon assessment of X-ray images is considered. The paper analyzes the requirements for some expert DSS and their main characteristics that they should have
Gas exchange readjustments in response to hypoxia and hypercapnia exposure in Magadan region military service draftees

Iressa V. Averyanova, Sergei I. Vdovenko

Our study identified gas exchange and external respiration characteristics during hypoxia and hypercapnia exposure in young men of the Magadan Region. A comprehensive survey in young men of military age, 18-21 yr., permanent residents of the Russia’s Northeast, was conducted.

Cognitive, emotional-affective, anxiety and autonomic disorders in patients with a new coronavirus infection (covid-19) in the acute period

Konstantin N. Melnikov, Viktor P. Kondratyev

The purpose of this work is to identify neuropsychiatric functions in patients at Department No. 1 responsible for medical care of patients with a new coronavirus infection at the Samara City Hospital No. 7. Appropriate scales and questionnaires were used for this purpose.

Photometric recorder of the carotid artery pulse wave with a system for stabilizing the clamping force

Alexey V. Berdnikov, Georgy V. Matrosov

Preventive monitoring, which increases the possibility of diagnosing and curing a disease at an early stage, is interesting and relevant in medical diagnostics. Recently, both minimally invasive and non-invasive diagnostic techniques have become widespread. They include the areas of cardiographic, encephalographic and myographic analyzers designed to study the state of the heart, the brain and striated muscles.

Impact of 3D transesophageal echocardiography assessment of mitral valve on short-term outcome of Balloon Mitral Valvuloplasty using additional quantitative parameters

Ibrahim Ahmed Elsawah, Ali Mohamed Al Amin, Abdel-Mohsen Moustafa Abdo, Mohamed Osama Taha, Essam Ahmed Khalil

The goals of this prospective observational study were to identify quantitative morphological factors that might aid in the prediction of PBMV outcome, as well as to define the function of 3D TEE in mitral valve evaluation. The trial comprised seventy individuals who had isolated severe rheumatic Mitral Stenosis (MS) who were scheduled for PBMV between October 2017 and March 2021.

Nutritional and biological value of natural-bio shoots mung bean “Mungoltin”. Food and biological values

Javokhir B. Khayitov, Guli I. Shaikhova, Dilshod D. Achilov, Munira J. Allaeva

Based on the results of our own research, examination of scientific dossier materials and reference literature data, it was established that dry powder Mungoltin made from the shoots of mung beans produced by Oriona-Scorpion LLC (Uzbekistan) contains a sufficient amount of protein, minerals, vitamins and dietary fiber, does not have a negative impact on the health status of experimental animals and does not result in functional and material cumulation.

Predictors of residual coronary artery disease after PPCI in diabetic patients with STEMI

Bassem Zarif

The present study consisted of 290 patients with ST-elevation myocardial infarction over one month period. About 50% of the patients with diabetes were more often female, with a greater prevalence of hypertension and dyslipidemia. In the diabetic group, the eGFR <60 ml/kg/min was associated with more residual CAD after PPCI and a higher residual SYNTAX score.

Development of modern technology for obtaining tinctures with sedative effect

Holida M. Yunusova, Zilola V. Turdieva, Nargiza B. Ilkhamova

The aim of this work was to experimentally select the optimal conditions for obtaining a tincture with an effective sedative effect consisting of a mixture of common motherwort grass, medicinal lemon balm and nettle leaves in a ratio of (1:1:1) and the development of quality criteria for the finished product.

Pre-angioplasty instantaneous wave-free ratio pullback predicts hemodynamic outcome in diffuse coronary artery disease

Ahmed Youssef Nammour, Hisham Mohamed Aboul-Enein, Mohammed Hamouda, Ahmed Atef Elhelaly

Serial stenoses or diffuse vessel narrowing hamper pressure wire–guided management of coronary stenoses. Characterization of functional relevance of individual stenoses or narrowed segments constitutes an unmet need in ischemia-driven percutaneous revascularization. To perform hemodynamic mapping of the entire vessel...
102 Features of the course of cardiovascular diseases against the background of COVID-19

Based on the analysis of 308 electronic medical records of patients with a confirmed diagnosis of a new coronavirus infection COVID-19, the features of the course of cardiovascular diseases at the regional level were studied.

107 The state of the cardiovascular system in women during pregnancy
Vakha A. Anzorov, Svetlana V. Moryakina

The work is devoted to the study of the impact of pregnancy on the state of the cardiovascular system in women. The process of pregnancy is accompanied by significant changes in the rhythm of the heart performance and blood pressure, and the duration of the ECG waves and segments are not significantly fluctuated. The heart rhythm, gradually increasing, reaches its maximum value of 87.3 beats per minute (P < 0.001) in the last trimester, in women of the reference group 69.8.

111 Prevalence of anxiety and depression disorders among medical students one year after the start of the COVID-19 pandemic
Aksana M. Kardangusheva, Diana A. Dzakhmysheva, Milana A. Kardanova, Amina V. Chanayeva, Marina A. Makaeva, Ilona S. Khagabanova

The aim is to study the incidence rate of anxiety and depressive disorders and their relationship with the everyday activity at the H.M. Berbekov Kabardino-Balkarian State University (KBSU) medical faculty students a year after the start of the Covid-19 pandemic. As a part of a cross-sectional observational study, a survey of 425 students at the Faculty of Medicine was conducted. The average age of the surveyed students was 20.9±2.1 years. The Spielberger-Khanin questionnaire, the HADS anxiety and depression scale, as well as a questionnaire specially developed by the authors to clarify some indicators of students’ activity were used.

119 Anatomical and topographical features of the gracilis muscle of the thigh from the position of using it for autotransplantation

Autotransplantation of a free flap of the gracilis muscle is currently actively used in reconstructive surgery for persistent prosoplegia, injuries of the brachial plexus, for plastic closure of defects in the upper lip, and in the treatment of pelvic sepsis. This study provides important information about the anatomical features of the gracilis muscle and its neurovascular bundle, which are highly variable.

124 Functional state of the students’ cardiovascular system under the conditions of mountain hypoxia
Vakha A. Anzorov, Svetlana V. Moryakina

The article presents the functional state of the cardiovascular system of students under conditions of high-altitude hypoxia. From the analysis of the results of our research, it can be argued that due to the fact that students live under conditions of a reduced oxygen content in the atmospheric air, there is a significant increase in the systolic output of their hearts. Thus, the systolic blood volume of students under the conditions of mountain hypoxia increases, reaching the maximum values of 63.4 ml (P <0.05) in males and 64.4 in females in the middle-level mountains.

130 Features of life activity and the incidence rate of anxiety and depressive disorders among medical students studying remotely during the epidemic of a new coronavirus infection (Covid 19)
Aksana M. Kardangusheva, Diana Z. Kaskulova, Maryam H. Kurdanova, Antonina F. Budnik, Olga V. Voronova, Zukhra A. Gelyakhova

The aim is to study the features of life and the incidence rate of anxiety and depressive disorders among medical faculty students studying remotely during the epidemic of a new coronavirus infection (Covid 19) at the Kabardino-Balkarian State University named after H.M. Berbekov. We examined 335 students (34% males, 66% females) at the Faculty of Medicine in November-December 2020.
Elena B. Kvakina,

Gallery of Great Scientists. Garkavi’s Core Team
Elena B. Kvakina is an outstanding scientist, a theorist, who devoted her life to the development of the original conceptual philosophy about the system of adaptational reactions by a human organism, a co-author of Scientific Discovery “Pattern of development of qualitatively differing general unspecified adaptational reactions of the organism” (Scientific Discovery Registration Certificate No. 158 issued by the Committee on Inventions and Discoveries at the Council of Ministers of the USSR, Moscow, 1975). Elena B. Kvakina was as a friend and colleague of Lyubov Kh. Garkavi, so they shoulder to shoulder went through the life path of promoting fundamental research to the top of scientific comprehension.

Elena B. Kvakina was born in 1933 in a family of priests: her great-grandfather was Fr. Terenty and her grandfather Fr. Aleksandr Ukolov, who worthily served the God and Fatherland, and their parents, Ardashev Boris I. and Maria A. Ukolova, were able to transfer to their daughter the best qualities of a scientist with their scientific feat. Having graduated as a Medical Doctor, Elena B. Ukolova, Mrs. Kvakina by marriage, started her activity at the Oncological Institute in Rostov-on-Don, where an experimental department headed by M.A. Ukolova began a huge research work. Fascinated by the idea of neuro-endocrine regulation of the human body during the development of malignant tumors, young enthusiastic experimenters Lyubov Garkavi, Elena Kvakina and Yuri Bordyushkov, premiered on the scientific stage after promotion by their leader, shone at most recognized international forums, arousing genuine interest of prominent scientists both in Russia and abroad. Following Hans Selye, they were able to understand, significantly deepen and advance the quantitative approach in assessing the adaptation activity of the human organism.

Of course, the genius L.Kh. Garkavi was the pace-maker in that core team of the keen researchers, but the phenomenal expertise, deep intelligence and labor dedication of Elena B. Kvakina made it possible to turn diamond mines into shining diamonds of great scientific works: the foundations of the theory of adaptation reactions were laid, and a fresh periodic system was built to show a tetrad of the same regular reactions of training, calm, elevated activation and stress at different levels of human body reactivity. Like D.I. Mendeleev, who saw his periodic system of chemical elements in a dream, Elena B. Kvakina’s dream offered the subcortical-cortical connections embodied in a multi-storey image of the multi-level development of adaptational reactions within a wide stimulus exposure range. The unpretentiousness of Elena B. Kvakina did not allow her to advertise her conjecture: especially since uninterrupted joint reasoning and mutual impregnation of the fabric of scientific thinking firmly integrated the triumvirate of Garkavi, Ukolova and Kvakina.

Concluding my brief essay about Elena B. Kvakina, it is necessary to restore her image of not only an outstanding scientist, but also of a very harmonious personality, combining brain training with a variety of sports - tennis, volleyball, cycling. The hearth, where her beloved daughter Tatyana and her husband Stanislav were, was always kept warm and filled with magnificent live piano music. So, upon passing the Torch of Great Science on to us, in the present issue of the Journal we are offering a lot of new things in the field of systemic medicine to our readers.

With sincere gratitude,

Alla I. Shikhlyarova
Professor, Doctor of Biological Sciences
The National Medical Research Center of Oncology
at the Ministry of Health of Russia
Addressing our current topics: Modeling of Pathological Processes

Modern medicine is now facing a huge number of challenging issues, which are associated with the present-day circumstances: the gap between rich and poor countries in public health care and a new virus pandemic with actualizing of vaccination so that all this unfavorable combination has become an obstacle in developing fundamental medicine areas like cardiology and oncology.

We cannot find latest developments of complex pathogenetic therapy of complicated or modified forms of common diseases (CVD, cancer, endocrine and immune pathology), and this shall be attributed to missing fundamental developments capable of revealing mechanisms of mutually-interrelated, cross-actions and -effects of different nosological states.

This mysterious puzzle in medical science attracts more and more researchers who deal with a complicated clinical pattern made by comorbidity against the background of a primary disease. Therefore we think it is reasonable to treat in this Journal issue some conceptual ideas on extremely complex mutual relationships between a malignant tumor growth and some accompanying diseases as comorbidities. The term comorbidity is used in epidemiology to indicate the coexistence of two or more disease processes. It is known that most cancer patients have often a long list of other disorders and diseases like hypertension, hypercholesterolemia, CVD, diabetes, osteoporosis, depression, chronic obstructive pulmonary disease (COPD) and thyroid diseases.

The question is how to read or properly interpret a highly sophisticated system like cancer in order to identify the factors modifying this malignant process? We think it is very expedient to apply modeling of combinations of “some driving forces” or “some driving factors” in a model system of the main process. By this means modeling different pathological processes, using the same testing subject, can be a key to an understanding of how complex inter-system relationships function.

The presented experimental research works make ambitious attempts to formulate the conceptual philosophy of the mechanism which determines the behavior of different types of a malignant tumor, including genetically modified cancer cells, under strictly defined conditions, namely, against the background of an accompanying disease in an organism: chronic pain or hypothyroidism, obesity or diabetes mellitus. The standardized profile in research Model-in-Model is opened due to an involvement of biochemical, electrophysiological, hormonal and morphological methods and techniques, the summarized results of which make possible to predict possible ways of control & regulation of tumor process, metastasizing, life expectancy and gender differences.

Our idea is to draw attention of our thoughtful readership to some papers published herein which treat modeling of pathological processes and reveal some salient features of actions and effects produced by comorbidities on a malignant tumor growth: we hope they will be useful and possibly can shine a light on many hidden mechanisms of control & regulation of key hemodynamics and cardiovascular changes found in the integrated homeostatic field in a human organism. The presented intriguing research works should invite and motivate other scientists to make next steps to further amazing fundamental studies.
Original Research

Mechanisms of cancellation by pain of genetically determined inhibition of a malignant tumor growth in experiment

Oleg I. Kit, Elena M. Frantsiyants, Alla I. Shikhlyarova*, Inga M. Kotieva, Irina V. Kaplieva

National Medical Research Centre for Oncology, Rostov-on-Don, Russia, 344037, Rostov-on-Don, 14 liniya, 63, building 8

* Corresponding author: 8(863)2001000-(482) shikhliarova.a@mail.ru

Abstract

Aims are to study the nature of the processes of carcinogenesis of experimental B16/F10 melanoma in uPA gene knockout mice modified by chronic neurogenic pain and investigate some electrophysiological mechanisms of melanoma development.

Materials and methods. We used 48 C57BL/6-PlautmI.IBug-ThisPlau6FDhu/GFDhu mice of both genders with urokinase gene knockout and 102 C57BL/6 mice of both genders with the normal genotype. Chronic neurogenic pain (CNP) was produced due to bilateral ligation of the sciatic nerve. Against the above background, all animals were transplanted with B16/F10 melanoma. To study the mechanism of CNP, studies of the intracellular electrophysiological activity of neurons of the central nervous system of the snail Helix pomatia in the body in vivo were carried out. CNP was reproduced by dosed pressing of four main nerves with Fresnel hairs that with time turned into increasing pain. Membrane potential (MP), action potential (AP) and firing rate (FR) parameters of intracellular bio-potentials of the command neuron RPaG3, continuously recorded using an ultrathin glass microelectrode for 4-5 days, were analyzed.

Results. It was detected that an activation of cancerogenesis during the modification of the progression of experimental B16/F10 melanoma in C57BL/6-PlautmI.IBug-ThisPlau6FDhu/GFDhu mice with uPA gene knockout using CNP is accompanied by a 2-fold acceleration in the time of tumor production, stimulation of the growth of the primary tumor nodes from 1.05±0.08 cm³ to 9.50±0.98 cm³ (p<0.001) and multiple metastasizing to the lungs, a reduction in the life span from 34.67±0.67 to 21.33±2.19 days (p<0.001) <0.05) in the genetically modified mice, by changing some gender-specific characteristics of the progression of the malignant process. The neuropathic nature of pain resulting from command neuron compression or ligation of the sciatic nerves is essentially identical to the implementation of genetic programs responsible for the control of life and death, reproducing events in carcinogenesis with the progression of a malignant tumor.

Conclusion. The initiation and chronization of pain at the local level of the nervous system can lead to generalization of the pain syndrome and contribute to the cancellation of genetically predetermined programs of carcinogenesis.

Keywords

Chronic neurogenic pain, Carcinogenesis, Urokinase knockout mice, Melanoma, Neural compression, Intracellular electrophysiological activity of neurons

Introduction

Genetically engineered mouse models (GEMM) have been successfully used for decades in human cancer modeling [1]. The use of the mice for this purpose can provide an appropriate platform for prospective studies to explore some specific hypotheses and causal associations in human diseases. In addition, continuous progress in genetic engineering allows achieving a more precise control of spatial and temporal genes, improving the ability to reproduce events in carcinogenesis and disease progression [2]. However, many etiological, genetic, and physiological factors must be considered to properly simulate a human disease in mice, especially for certain types of cancer.

Metastatic cutaneous melanoma is inherently resistant to most conventional chemotherapeutic agents, and in this case the patient survival rates are usually poor. Identifying the key elements of the melanoma development and their interactions is critical to offering more effective prevention and treatment strategies. The stages of development of melanocyte precursors in humans and mice are largely similar, which makes mice suitable for modeling human melanocytic pa-
thology. Moreover, modern genetic engineering technologies make it possible to flexibly control multiple genetic alleles independently of each other that make it possible to build a model for testing a multi-factor hypothesis [3].

The “urokinase-type plasminogen activator”, or simply “urokinase” (uPA), is a key serine protease involved in the conversion of inactive plasminogen to active plasmin, which in its turn is functioning in a number of carcinogenesis events. Several studies have demonstrated the involvement of the uPA–uPAR system at the early stages of tumor formation. For example, melanoma progression has been impaired in uPA-deficient mice [4].

It is considered proven that chronic pain is not a symptom of a disease, but it is an independent pathology requiring its proper study and treatment [5]. This is confirmed by the data of the electrophysiological component of the mechanism of chronic neurogenic pain because of its long-term pathological low-threshold actions and effects [6]. Pain reduces the body’s resistance to the development of malignant tumors; it is one of the most constant symptoms found in cancer patients, and with a progressive course of the disease, its occurrence rate increases [7, 8].

In the present research work, we aimed to study the nature of the oncological process during the modification of the carcinogenesis of experimental B16/F10 melanoma in uPA gene knockout mice using chronic neurogenic pain and investigate some of the mechanisms of its development.

Materials and methods

Animals

In the experiment, mice of both genders (n=48) of the C57BL/6-Plautm1.1Bug-ThisPlau6FDhu/GFDhu line were used with an initial individual weight of females of 24-26 g, males of 31-33 g, having a knockout in the urokinase gene, delivered by the Laboratory Animal Breeding Center “Pushchino”, Branch of the Institute of Bio-Organic Chemistry named after Academicians M.M. Shemyakin and Yu.A. Ovchinnikov (Pushchino, Moscow Region). The characteristics of the C57BL/6-Plautm1.1BugThisPlau6FDhu/GFDhu animals were the following: coat color: black, modification method: target mutation (knockout) to produce a protein (uPA) that is incapable to bind to the urokinase-type plasminogen activator receptor. Mutant animals may be used in studies of chronic tissue inflammation, mechanisms of fibrinolysis, oncogenesis, and vascular growth in tissues.

Also, mice (n=102) of both sexes of the C57BL/6 line (n=168), aged 8 weeks, with an initial individual weight of females of 21-22 g and males of 28-30 g were used, delivered by the Federal State Medical & Biological Institution “Research Center of Biomedical Technologies” (Branch Andreevka, Moscow Region).

Laboratory animals (mice) were kept under natural light conditions with free access to water and food. Work with animals was carried out in accordance with the rules of the Directive 86/609/EEC on the Protection of Animals Used for Experimental and Other Scientific Purpose, as well as in accordance with the International Guiding Principles for Biomedical Research Involving Animals and Order No. 267 “Approval of the Rules of Laboratory Practice” dated June, 19, 2003 issued by the Ministry of Health of the Russian Federation.

Model of chronic neuropathic pain

The model of chronic neurogenic pain (CNP) was reproduced by applying a ligature to the sciatic nerve from both sides under xylazolethyl anesthesia [9]. Anesthesia: xyl-zoletyl, 10 minutes before the main anesthesia; premedication: xylazine (the Xila preparation) intramuscularly, at a dose of 0.05 ml/kg of body weight (according to the instructions), then 10 minutes later, Zoletil-50 was administered at a dose of 10 mg per 100 g of body weight. In sham-operated animals, the nerve was exposed but not ligated. On post-surgery day 14, mechanical allodynia and hyperalgesia were measured.

To study the mechanism of development of neurogenic pain, we selected functionally active neurons of the central nervous system of the grape snail Helix pomatia as a model of an integral part of the organism in vivo. We used the electrophysiological technique by V.I. Orlov for continuous recording of intracellular bio-potentials of neurons before and after the application of a long-term low-threshold action (LTA) with dosed pressing of the four main nerves, which eventually turned into increasing pain [6,10]. For the purpose, the Fresnel hairs of a selected diameter were utilized, which provided a constant low level of the nerve compression after switching on the LTA mode. In order to minimize the impact on the nervous system, we recorded and analyzed the electrophysiological pa-
rameters of only one identified RPaG3 command neuron: membrane potential (MP), action potential (AP) and firing rates (FR) of intracellular bio-potentials of the neurons, which were supplied using an ultrathin glass microelectrode, filled with electrolyte. Additional devices ensured the viability of the animal, the maintenance of the required comfortable temperature, and the supply of saline with nutritional components at a strictly constant rate.

After the production of chronic neurogenic pain, upon expiration of 2 weeks, the B16/F10 melanoma cells were transplanted into C57BL/6-PlautmI.IBug-ThisPlau6FDhu/GFDhu mice of both genders by a standard subcutaneous injection of the tumor suspension into the right shoulder blade area in a volume of 0.5 ml of cell suspension at a dilution of 1:10 in physiological solution. The tumor strain of mouse melanoma B16/F10 was supplied by the Russian National Medical Research Center of Oncology named after N.N. Blokhin at the Ministry of Health of Russia.

For the purpose of experiment, mice of the C57BL/6-PlautmI.IBug-ThisPlau6FDhu/GFDhu line of the corresponding sex, transplanted with B16/F10 melanoma at the same dose and volume, but without chronic neurogenic pain were used as Reference 1.

The C57BL/6 mice of the corresponding sex with transplanted B16/F10 melanoma at the same dose and volume as it was the case with C57BL/6-PlautmI.IBug-ThisPlau6FDhu/GFDhu mice were involved as Reference 2.

The animals were decapitated 4 weeks after the inoculation.

The required statistical analysis of the results was performed using Statistica 10.0, and the data were presented as mean ± standard error of the mean. Data were analyzed using t-test, one- or two-way analysis of variance ANOVA (depending on the situation).

**Results**

The life span of mice of different lines with the growth of B16/F10 melanoma was identical: the average life time in males was recorded to be 1.5 times (p<0.05) less than that in the females. CNP did not affect the life span in the C57BL/6-PlautmI.IBug-ThisPlau6FDhu/GFDhu male mice, as compared with the C57BL/6 males (a decrease in the life span), while in all females, regardless of their genome, the life span under the influence of CNP was reported to be shorter (see Table 1 and 2 herein).

**Appearance of primary tumor nodes.** In the reference mice with the modified genome, the primary tumor was palpable from the first week of carcinogenesis, however it was detected as a pea-sized lump, while in the reference mice of the C57BL/6 line, the primary tumor appeared a week later, and its average volume almost immediately reached 1.0 cm$^3$ (see Table 1 and 2 herein).

Under the influence of CNP, the timing of the appearance of primary tumors in the mice with the normal genome and the mice with the knockout in the uPA gene changed in different directions: in the first

<table>
<thead>
<tr>
<th>Mice with a normal genome</th>
<th>uPA knockout mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>melanoma + pain</td>
<td>melanoma</td>
</tr>
<tr>
<td>Mice, n</td>
<td>22</td>
</tr>
<tr>
<td>Date of appearance of the tumor (day)</td>
<td>9.80±0.82</td>
</tr>
<tr>
<td>Average tumor volume (cm$^3$)</td>
<td></td>
</tr>
<tr>
<td>Week 1</td>
<td>0.06±0.01</td>
</tr>
<tr>
<td>Week 2</td>
<td>1.27±0.37</td>
</tr>
<tr>
<td>Week 3</td>
<td>5.91±1.48$^b$</td>
</tr>
<tr>
<td>Week 4</td>
<td>7.94±2.10$^b$</td>
</tr>
<tr>
<td>Metastases</td>
<td>lungs</td>
</tr>
<tr>
<td>Hemorrhages</td>
<td>–</td>
</tr>
<tr>
<td>Life span (days)</td>
<td>22.15±1.82</td>
</tr>
</tbody>
</table>

Notes: $^*$statistically significant difference compared with the growth of melanoma without pain, $^+$statistically significant difference compared with mice with normal genome, $^{1,2,3}$statistically significant difference compared with the growth of melanoma after 1, 2, 3 weeks of carcinogenesis.
case, the tumor appeared a week earlier than that recorded in the reference group; in this case in the males its initial sizes were much (11.7 times) smaller than those identified in the females, and they did not reach a volume of 1.0 cm³; in the second case, melanoma appeared on average 4 days later than in the reference animals, and its sizes were more than 1.0 cm³ (see Table 1 and 2 herein).

**Dynamics of primary tumor nodes.** In the uPA gene knockout mice, the melanoma growth dynamics depended on the gender. In the males, the tumors were characterized by a sufficiently active growth, and their sizes at week 4 of carcinogenesis did not differ from the values recorded in the mice with the normal genome: at week 2 of carcinogenesis, the tumor increased sharply (160.0 times) in its volume; at week 3, it did not statistically significantly change, but at week 4 it gave a second growth spurt, exceeding the previous level by a factor of 3.1 (see Table 1 herein).

In the females, tumors practically did not grow, and their sizes were minimal: by week 4 of carcinogenesis, its volume did not exceed 1.0 cm³ (see Table 2 herein).

CNP erased the gender differences in the development of melanoma in the C57BL/6-PlautmI.IBug-ThisPlau6FDhu/GFDhu mice, enhancing the growth of the primary tumors from week 2 to week 4 of carcinogenesis in all mice, regardless of their sex. As a result, in the mice of the C57BL/6-PlautmI.IBug-ThisPlau6FDhu/GFDhu line with CNP, the sizes of the primary neoplasms were statistically significantly larger than those found in the corresponding reference group: in the males at week 3 of carcinogenesis, they were 2.8 times greater and at week 4 of carcinogenesis, they were 1.3 times larger (p<0.05); in the females at week 3 of carcinogenesis they were 144.0 times larger, and at week 4 of carcinogenesis the sizes were 8.8 times greater; at the same time, the volumes of the tumors at week 4 of carcinogenesis were more than 7.5 cm³ both in the males and the females (see Tables 1, 2 herein).

The growth dynamics of the primary tumor node in the C57BL/6 mice, with the exception of some minor nuances, was almost the same both in the males and females: the tumor increased in its volume until their death: without pain exposure up to 4 weeks of the malignant growth and against the background of CNP up to 3 weeks; the size of tumors at week 3 of carcinogenesis did not depend on the presence or absence of CNP (see Tables 1, 2 herein).

Hemorrhages in the internal organs were typical only for the uPA knockout mice: in the males without pain exposure, they were localized in the lungs and the heart; in all mice with CNP, regardless of the gender, they were found in the lungs, while in the males thy- mus involution was recorded; the exception was the females without CNP, who had no hemorrhages (see Table 1 and 2 herein).

**Features of metastasizing.** Only in the females with the genetic knockout of uPA, single lung metastases were visualized, while in the males no melanoma metastasizing was found. CNP stimulated metastasiz-

### Table 2

<table>
<thead>
<tr>
<th>♀ melanoma</th>
<th>Mice with a normal genome</th>
<th>uPA knockout mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mice, n</td>
<td>melanoma +pain</td>
<td>melanoma</td>
</tr>
<tr>
<td>22</td>
<td>28</td>
<td>12</td>
</tr>
<tr>
<td>Date of appearance of the tumor (day)</td>
<td>10.15±0.98</td>
<td>5.29±0.20</td>
</tr>
<tr>
<td>Average tumor volume (cm³)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 1</td>
<td>-</td>
<td>0.70±0.25</td>
</tr>
<tr>
<td>Week 2</td>
<td>0.85±0.12</td>
<td>1.65±0.27</td>
</tr>
<tr>
<td>Week 3</td>
<td>2.75±0.731</td>
<td>2.50±0.49</td>
</tr>
<tr>
<td>Week 4</td>
<td>4.69±0.862</td>
<td>-</td>
</tr>
<tr>
<td>Metastases</td>
<td>spleen</td>
<td>heart, lungs, liver, uterus</td>
</tr>
<tr>
<td>Hemorrhages</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Life span (days)</td>
<td>30.25±1.67</td>
<td>19.17±1.35</td>
</tr>
</tbody>
</table>

Notes: *statistically significant difference compared with the growth of melanoma without pain, +statistically significant difference compared with mice with normal genome, 1,2,3 statistically significant difference compared with the growth of melanoma after 1, 2, 3 weeks of carcinogenesis.
ing, and in this case in the males melanoma began its metastatic dissemination to affect the lungs and the liver, while in the females, the number of metastatic spots in the lungs increased (see Table 1 and 2 herein).

In the mice with the normal genome, the metastasis loci depended on their gender: in the males without pain exposure, metastases were detected in the lungs, and in the females without pain, they were revealed in the spleen (see Table 1 and 2 herein). CNP contributed to spreading of metastatic lesions to other organs: in the males the spleen was metastasized, and in the females the heart, the lungs, the liver and the uterus were affected by the tumor cells.

Thus, the pathogenesis of B16/F10 melanoma in the C57BL/6-PlautmI.IBug-ThisPlau6FDhu/GFDhu mice with uPA gene knockout was accompanied by the early appearance of the primary tumor nodes and pronounced gender-specific characteristics: we observed in the males the “jump-like” growth dynamics of the primary tumors with 2 volume increase peaks at week 2 and 4, with hemorrhages in the lungs and the heart, but with no metastasizing; we found in the females no clearly-cut growth dynamics of their primary tumors (with their maximum at week 4, no more than 1.0 cm³), with isolated metastases in the lungs, however with no hemorrhages in the internal organs. CNP stimulated the growth of the primary tumor node and metastasizing in the genetically modified mice, increased the period of the appearance of the primary tumors, reduced life spans in the females only, while a greater number of the males, compared with the normal ones, lived longer than 3 weeks.

Revisiting the mechanism of CNP influence on B16/F10 melanoma progression

The experimentally discovered effects of the tumor growth stimulation under CNP left open the question of the primary neurogenic mechanism initiating the pathological dominant as an important condition for weakening the body’s resistance and, conversely, for a favorable realization of the pathogenesis of melanoma B16/F10 in the C57BL/6-PlautmI.IBug-ThisPlau6FDhu/GFDhu mice with the uPA gene knockout. It has been precisely chronization of pain generated by the long-term mechanical compression of the neurons is responsible for the changes which develop in the pathogenesis of the tumors in the animals with the disturbed nervous regulation. We tried to get closer to an understanding of the intimate mechanisms of neuron response patterns using the CNP models.

It is generally accepted that, despite the diversity and different complexity levels of the nervous system, all nervous systems are governed by the same general laws at the level of the morphological, physiological and biochemical activity of a nerve cell, the intra-neuronal organization of the methods and mechanisms of information transmission [11]. After establishing a stable operating mode of the neuron, the LTA mode was switched on. To understand the nature of the phase transitions of the functional state (FS) of the neuron, structuring into stages was undertaken that correlated with the level of pain in accordance with the known laws of electrophysiology.

As a result, it was found that at stage 1, when the LTA mode was turned on, the nature of the electrophysiological parameters of the background state of the neuron did not change immediately and for a long time, and the usual type of communication with other neurons continued (see Figure 1.1 herein). Noticeable changes in the functional state of the neuron began to appear at the 2nd stage, upon exposure approximately for 10–14 hours to the LTA mode. The spontaneous neuron activity with an adequate activation on EPSP and rest intervals turned into a rhythmic pacemaker mode, which gradually became dominant in the neuron activity (see Figure 1.2 herein). At stage 3, this endogenous hyperpolarizing wave (EHP) completely locked the AP generation, and after the action of EHP at that stage, the firing rate of AP generated by the neuron decreased, giving the neuron a break (see Figure 1.3 herein). This should be treated as the most important factor of the neuron survival program.

1–1.5 hours after stage 3, the rhythmic AP generation was interrupted by pulses of small amplitude of EHP of the membrane, creating a pause in the AP generation. EHP at stage 4 kept the membrane potential of the neuron at the upper limit of its functional norm. There were pulsations of the functional state of the neuron (see Figure 1.4 herein). At stage 5, pain could be characterized as an exhausting severe factor with the development of central sensitization. The membrane potential of the neuron was depolarized by a more intense synaptic influx. The high-amplitude III-activity (Editorial note: for more info please visit electrophysiological-evidence.pdf (cardiometry.net) Valery I. Orlov, Alla I. Shikhlyarova. Intracellular electrophysiological evidence: how pain is experienced by neurons. Cardiometry; Issue 17; November 2020; p. 8-21) appeared. The law of the membrane electro-
physiology should be applicable, according to which higher-amplitude HP waves caused stronger output effects, an increase in the AP firing rate upon the action of EHP (see Figure 1.5 herein).

At stage 6, recorded for more than 3 hours, depolarization of the neuron membrane occurred, which constantly increased. The high-amplitude EHP waves made attempts to return the MP level to the normal one, but at the same time the growing excitatory exogenous influx caused by the irradiation of sensitization processes methodically increased the MP depolarization (see Figure 1.6 herein).

At stage 7, the depolarization of the neuron membrane increased more and more. The mode of operation of the neuron became extreme, close to its critical condition. Due to a great thunderstorm of incoming excitatory signals, the neuron programs were started, striving to transfer it to its off-state. It is known that a neuron ceases to generate spikes when its membrane is extremely hyperpolarized or depolarized. At this stage, one of its running programs produces powerful pulses to depolarize the neuron’s membrane. Membrane depolarization increases the firing rate of generated APs (see Figure 1.7 herein).

At this time, the critical phase of the execution of two competing programs takes place: one of them is targeted at switching-off, while another is aimed at keeping life. The membrane in the circumstances is extremely depolarized. In other words, the difference in the electrical potential of the membrane between its outer and inner surface is very small. The AP generation stops. High-frequency EHPs of small amplitude gradually fade. There are no pulses available (see Figure 1.8 herein). The program, which runs in synergy with the exogenous excitatory potentials, is the winner. The membrane generator mechanisms are switched off. Intracellular electrical potentials from the neuron are not recorded. Signs of the absence of

Figures 1.1-8. show stages of neuron response to LTA. General view of the final process (experimental data obtained by Orlov VI., 2020).
vital activity of the neuron are evident. The recording line coincides with the isoelectric line.

According to an illustrative definition proposed by V.I. Orlov, the life of the neuron is ended with “a guitar string breaking sound”, i.e. high-frequency decaying oscillations similar to that when a guitar string breaks, and the typical sound can be heard, but no further music is possible [6].

So, a gradual change in the functional state of the CNS command neuron was traced during long-term action produced by a low-threshold pain factor, and a complex oscillatory dynamics of key electrophysiological parameters of MP, AP, IAF was revealed, starting from the initial time without LTA until the time of loss of response by the neuron and, in fact, turning off the neuron to leave its active state under the continuing LTA (see Figure 2 herein).

Figure 2. Dynamics of passing the point of no return by the command neuron in the central nervous system (according to V.I. Orlov).

Discussion

There is only one answer to the question of how the modes of hyper- and hypopolarization of the neuron membrane are switched over from one to another under chronic pain: the regulator of the life program is the internal structures of the cell, and the main regulator is DNA operating via the RNA messengers. DNA continuously receives information about the functional state of the membrane and about the incoming signals, which change its potential, and issues commands to turn on or off certain ion channels in the membrane, changing the mode of its operation, executing the life program. DNA of each neuron has its own specialization, somewhat different from the others. A highly rich branching of the dendritic tree provides communication between the neurons with the use of APs, performing coordination between the elements in an organism [12]. In addition, there are some other channels of energy–informational communication between the DNA in the cells and the external environment of the organism [13,14]. Due to the interconnection and exchange of information, the cells of the neural network having a high level of their self-organization act not as chaotically operating units, but as an integrated, well-coordinated, mechanism, providing the proper formation of integral adaptation reactions by the organism, within the framework of which the actions and effects made by factors of low intensity are realized [15-18].

Despite the difference in the space-time framework of the course of CNP at the cellular levels of the neuron and the organism as a whole, we have noted some common key points and connecting threads in synergy of both processes. Firstly, this was indicated by the similarity in the phenomenon of the unchanged neuron parameters at the first stage of the study and the absence of any signs of an accelerated tumor growth at week 1. Secondly, the dynamics of the subsequent processes showed a sharp increase in the volume of melanoma at week 2, which was reinforced by a burst of the high neuron activity at stages 2–3 in the form of the dominance of the pacemaker regime and the development of an endogenous hyperpolarization wave locking the AP generation. But it has been precisely this important event of reducing the AP firing rate that has given the neuron an opportunity to rest in order to survive. This fact was reflected in the discontinued tumor growth at week 3, when the volume of the tumor remained almost unchanged. This sort of synchronism was noted at further stages, when a high-amplitude firing began at the level of the neuron at stage 4–5 due to the ongoing synaptic inflow, which depolarized the neuron membrane, canceling the effect of hyperpolarization. At the same time, we observed the stimulated tumor growth again, to the extent that the gender-specific differences in the mice with the genetic knockout for urokinase disappeared. In parallel, at the final stages of the chronic pain stimulation of the neuron, the program of switching off the genetically determined generator mechanisms of pulse activity won, and the death of the command neuron was recorded under the continuously applied LTA involving the neural network for spreading of
the latter. It becomes obvious that at the final stage of melanoma development, the genetically determined inhibition of the progression of the malignant tumor, which forms multiple metastases in the lungs, accompanied by hemorrhages, involution of organs, is also canceled, resulting in accelerated death of the animals. It is accompanied by changes in different indicators and parameters at different hierarchical levels in an organism [19].

Conclusion

Thus, the revealed experimental parallel lines are very useful to properly understand that the activation of the oncological process under the modification of the carcinogenesis of experimental B16/F10 melanoma in the C57BL/6–Plau6/F10uPA−/−Plau6/F10uPA−/− mice with uPA gene knockout under CNP has the centralized neurogenic nature and covers different hierarchical levels, from the genomic level to the level of the organism. The neuropathic nature of pain, produced by the command neuron compression or ligation of the sciatic nerves, is essentially identical to the realization of the life control and death programs, because of reproducing the essential key events in carcinogenesis and progression of a malignant growth.

In other words, the initiation and chronization of pain at the local level of the nervous system is capable of generalizing the pain syndrome, on one hand, and, on the other hand, it can contribute to the inversion of genetically predetermined programs of carcinogenesis. That has been confirmed by the acceleration in the production and stimulation of the growth of the primary tumor nodes and metastasizing, the decrease in life spans in the genetically modified mice, and the change in the gender characteristics of the malignant process progression.

Statement on ethical issues

Research involving people and/or animals is in full compliance with current national and international ethical standards.

Conflict of interest

None declared.

Author contributions

The authors read the ICMJE criteria for authorship and approved the final manuscript.
Relationship between comorbid pathology and tumor progression. Morphological portrayal of internal organs in modeling the growth of Guerin’s carcinoma under diabetic conditions


National Medical Research Centre for Oncology, Rostov-on-Don, Russia, 344037, Rostov-on-Don, 14 liniya, 63, building 8

* Corresponding author: 8(863)2001000-(482) shikhliarova.a@mail.ru

Abstract

**Topicality.** An increase in the incidence of malignant tumors progressing against the background of various comorbid pathologies determines the need to study the mutual influence of pathological processes using experimental modeling. Such models, for example, can reproduce the tumor growth modified by the comorbid condition of diabetes mellitus, the incidence rate of which is now increasing exponentially. In this case, a clear demonstration of changes in the structure of organs outside the zone of the primary tumor node, using data on the direct growth of the tumor and the content of diabetes markers therein and in the perifocal zone, can serve as an evidence-based argument for the implementation of the mechanism of modified tumor progression.

The aim of our research work is to assess the state of the histological structure of some internal organs (the kidneys, the ovaries, the peritoneum) when modeling the main pathological process in animals, the growth of Guerin’s carcinoma, against the background of a comorbid state of experimental diabetes.

**Materials and methods.** We used 32 outbred male and female rats weighing 180–220 g to reproduce the model of experimental diabetes by a single intraperitoneal injection of alloxan at a dosage of 150 mg/kg of body weight. One week after the production of persistent hyperglycemia in the range of 25.4±1.2 mmol/l, the animals were transplanted with Guerin’s carcinoma subcutaneously in the region of the right shoulder blade. Upon expiration of 2 weeks, the animals were decapitated, and the harvested organs were prepared according to the practice stages of morphological preparation for staining sections with hematoxylin-eosin, followed by morphological examination of the structure with the use of the Leica DM LS2 microscope with the Olympus optical. C-5050 Zoom video camera and the Mofotest software. Photographing was carried out with magnification x10, x40, x100.

**Results.** Our study of the morphological portrayal of the ovary, the kidney, the visceral and parietal peritoneum bears witness to the identity of the changes, consisting in a total metastatic lesion and abnormal transformation of the normal structure of all the studied organs only in the female rats, when modeling the comorbid state of diabetes mellitus. At the same time, the aggressive nature of the tumor progression was manifested in the blood filling of the vessels and hemorrhage, followed by the release of tumor cells, their settlement, the enhanced proliferation, the formation of strands and compaction of cell aggregations throughout the volume of the organ. Some gender specific features of the tumor progression were noted, which were found in the female rats along the path of active metastasizing in case of small primary tumors, and in the male rats along the path of stimulating the growth of the primary focus without metastasizing. It was revealed that these differences are associated with different degrees of saturation of the tumor and perifocal zone with glucose, and they are determined by the state of the insulin/insulin-like growth factor (IGF) axis.

**Conclusion.** Morphological examination of the organs affected by metastatic Guerin’s carcinoma in the female rats with diabetes mellitus makes it possible to detect not only the synergy of both pathological processes, but also a powerful pro-oncogenic effect of the comorbid state of diabetes in the implementation of the tumor growth program.

**Keywords:** Guerin’s carcinoma, Diabetes mellitus, Comorbid pathology, Organ morphology, Female and male rats, Gender differences

**Imprint**

Introduction

Currently, there is no doubt about the relevance of the problem of the influence of comorbid conditions on the processes of carcinogenesis, since the role of the micro- and macro-environment in the malignant transformation of cells is known [1-3]. Diseases associated with cancer, having their own “face”, increase the diversity of the tumor diseases, and this pathological consolidation contributes to the weakening of the defense systems and a significant decrease in the antitumor resistance of the body. This is of decisive importance at the level of the whole organism, and only technologies of integrative activation therapy can help improve the situation to a certain extent by purposefully increasing nonspecific resistance [4–6]. An increase in the incidence of malignant tumors progressing against the background of various comorbid pathologies determines the need to study the mutual influence of pathological processes using experimental modeling [7].

As to the scale of modern medical problems, such as the growth of oncolgical, cardiovascular diseases, the pandemic of coronavirus infection, there is a significant increase in the incidence of diabetes mellitus (DM), which is one of the most topical pathologies that entails profound metabolic shifts at different levels of a self-organization organism: from the molecular to the systemic one. The clinical course of DM is accompanied by the emergence of many risk factors: the development of nephropathy and retinopathy, the determination of multiple vascular complications, up to the coronary, carotid, and peripheral vessels [8,9]. The main pathological changes in DM affect the biochemical environment of the body and are characterized by dysregulation of energy metabolism (glucose, amino acids and fatty acids), insulin resistance, rapid development of oxidative stress, and inflammation. This leads to various complications and entails functional and morphological damage to target organs, such as the heart, the kidneys, the liver, the eyes, the reproductive and other organs [8,9].

It should be noted that among the many mechanisms of the pathogenesis of diabetic complications, an important key component is oxidative stress mediated by reactive oxygen species (ROS) [9]. Normally, it is ROS that are required to maintain cellular signaling, as well as trigger antioxidant reactions in response to stress. When ROS levels become excessive, as it is the case with diabetes, there are dangerous consequences for the body. In DM, elevated and persistent levels of ROS cause a direct effect on the integrity of mitochondria and reduce the translational ability of cell signaling, which contributes to a decrease in tissue integrity and pathological transformation of organs [9].

Both fundamental and clinical data confirm the relationship between DM and a malignant process, since patients with malignant tumors often have diabetes [10]. The implementation of the biological links between DM and the malignant process includes hyperinsulinemia, hyperglycemia, chronic inflammation, often caused by obesity, as was pointed out about half a century ago by V.M. Dilman. [11]. Although the strongest relationship between these pathologies is in the pancreas and liver, there are many other organs involved in carcinogenesis in patients with DM, including the breast, the endometrium, the bladder, and the kidneys [12].

Thus, the vicious circle formed by organ pathology, being included in carcinogenesis as a comorbid condition, becomes a matrix for the development of a malignant process. It is of considerable interest to observe the development of a tumor disease and the behavior of the cell population of the tumor in the territory of organs that were not initially affected by the tumor transformation against the background of concomitant pathology of DM.

The aim of this work is to assess the state of the histological structure of some internal organs (kidneys, ovaries, peritoneum) when modeling the main pathological process in animals, the growth of Guerin’s carcinoma, against the background of a comorbid state of experimental diabetes.

Materials and methods

Information about the animals and their keeping

The studies were carried out on outbred male and female rats (n=32) 180-220 g. The animals which originated from the stock kept by the Federal State Medical & Biological Institution “Research Center of Biomedical Technologies” (Branch Andreevka, Moscow Region) were kept under natural lighting conditions with free access to water and food. The work
was carried out in accordance with Directive 86/609/EEC on the Protection of Animals Used for Experimental and Other Scientific Purpose as well as in accordance with the “International Recommendations for Biomedical Research Using Animals” and Order No. 267 “On approval of the rules of laboratory practice” dated 19 June 2003 issued by the Ministry of Health of Russia. The study record was approved by the Commission on Bioethics of the National Medical Research Center of Oncology of the Ministry of Health of Russia on 01.09.2020, record No. 21/99 prepared by the Ethical Committee. Animals were manipulated in a box in compliance with the generally accepted rules of asepsis and antisepsis. Animals were decapitated with a guillotine 14 days after transplantation of Guerin’s carcinoma and reproduction of experimental diabetes.

Modeling of malignant growth

We used a strain of Guerin’s carcinoma, delivered by the Federal State Budgetary Institution “National Medical Research Center of Oncology named after N.N. Blokhin” at the Ministry of Health of Russia. Material for transplantation was harvested from donor rats on days 12-16 of tumor growth. Transplantation of Guerin’s sarcoma was carried out by standard subcutaneous injection of 0.5 ml of a suspension of the Guerin’s tumor cells in a 1:5 dilution with saline solution under the right shoulder blade. The distribution of animals into groups was carried out by random sampling: the intact group (n=8), the reference group with diabetes (n=8), the comparison group (n=8) rats with standard subcutaneous transplantation of Guerin’s carcinoma, the main group (n=8) rats, which were first reproduced with diabetes, and then, 1 week after achieving persistent hyperglycemia, were transplanted with Guerin’s carcinoma.

Modeling the comorbid state of diabetes

To reproduce experimental diabetes, alloxan was intraperitoneally injected once at a dose of 150 mg/kg of body weight. Then during the week we measured the content of glucose in blood. The high content of glucose in blood, in the range of 15-30 mmol/l, indicated the reproduction of diabetes mellitus. At the time of transplantation of Guerin’s carcinoma in animals of the main group (n=8), the average blood glucose was 25.4±1.2 mmol/l, while in the group of intact animals (n=8) it was 5.2±0.3 mmol/l.

Morphological studies of internal organs in female rats

After decapitation of the animals, the organs (ovaries, kidneys, and peritoneum) were isolated, fixed in 10% neutral formalin, followed by buffing and embedding in paraffin. Paraffin sections of organs were stained with hematoxylin-eosin. For microscopy of preparations, a computer optical complex based on the Leica DM LS2 microscope with the Olympus optical C-5050 Zoom video camera and the Morfotest software was used. Photographing was carried out with magnification x10, x40, x100.

Results

Previously, it was shown that DM in males stimulated the growth of transplanted Guerin’s carcinoma, as a result of which the tumor volumes after 15 days were 75.1±6.7 cm³ that was 1.8 times (p<0.05) higher than in the reference group, equal to 40.7±3.9 cm³. In the females on the background of DM, already after 10 days, the dynamics of tumor growth was opposite and the subcutaneous component of the tumor, equal to 15.98±1.6 cm³, was 1.5 times less (p<0.05) than in the reference group where the average volume tumor size was 23.6±2.5 cm³. After 15 days, the difference between the groups persisted, and the tumor volume in the main group in the females was 1.3 times less than that in the reference group (39.35±3.8 versus 50.4±5.0 cm³, p<0.05), however, metastatic lesions of internal organs: the ovaries, the kidneys, the parietal and visceral peritoneum were found [8].

First of all, it was important to understand the root cause of the gender differences in the modification of DM processes of carcinogenesis. The data of biochemical and enzyme immunoassays made it possible to establish that the modeling of DM in females causes an increase in the glucose level by 1.8 times (p<0.05) in the tumor, while in the perifocal zone the glucose content literally rolls over, increasing by a factor of 8.1 versus the reference group. At the same time, multidirectional changes in the content of insulin-like growth factor 1 (IGF-I) were revealed as follows: an increase by a factor of 6.3 in the tumor and a decrease by a factor of 3.2 in the perifocal zone, as a result of which, apparently, with small volumes of primary node, the tumor became more “aggressive” and actively metastasized.

On the contrary, induced DM in the males reduced the levels of glucose, insulin-like growth factor 2 (IGF-II), and insulin-like growth factor-2 (IGFBP-2) carrier protein in the carcinoma itself by a factor of 8.4, by a
factor of 3.1, and by a factor of 1.7 (p<0.05), respectively. At the same time, the content of IGF-I and IGFBP-2 in the tumor increased 1.4 times (p<0.05) and 1.3 times (p<0.05), respectively, without changing the glucose concentration in the perifocal zone. As a result of this transformation of the biochemical environment, the volumes of tumors increased significantly and exceeded those with the standard growth of Guerin’s carcinoma, but metastasizing to the visceral organs was not observed.

Since the locally inhibited growth of Guerin’s carcinoma in the female rats under the conditions of DM acquired a pronounced metastatic character, it was necessary to carry out morphological examination of some internal organs and assess the degree of damage. Our histological analysis of such internal organs as the ovaries and kidneys, as well as the visceral and parietal peritoneum, revealed common signs of aggressive capture by tumor cells and the full loss of the architectonics of the organs. First, attention was drawn to the significant blood supply to large and small blood vessels, in the gaps of which one could see tumor cells. Apparently, the pathogenic effect of diabetes on the cardiovascular system, to a large extent, initiated metabolic disorders, leading to changes in the permeability of the vascular wall and hemorrhages, which contributed to the colonization of tumor cells. Secondly, in significant areas of various organs under study, one could see the formation of powerful strands of tumor cells, forming dense conglomerations, filling the entire parenchymal space. The morphological picture of the tumor lesions of the tissue of the ovary, the kidney and the peritoneum was analyzed.

Visual examination of the ovarian tissue made it possible to detect an almost complete tumor replacement of the parenchyma of the hormone-producing organ with the loss of the structure of follicles from primordial to mature. At the same time, it was often possible to identify false follicle-like structures formed by the tumor cells themselves as an adaptation to the loss of the dominant function of the affected organ. The presented histological material reflects the main key points of the process of the secondary intraorganic generalized growth in the ovary of carcinoma, which is initially transplanted under the skin of an animal under the conditions of diabetes (see Figures 1–4 herein).

Figure 1. Examples of blood filling and hemorrhage with the colonization of tumor cells in the ovarian tissue in female rats when modeling the growth of Guerin’s carcinoma against the background of diabetes mellitus. Stained with hematoxylin-eosin, magnified x100.
Morphological portrayal of the kidneys

In the rats with Guerin's carcinoma transplanted against the background of the metabolic disorders induced by diabetes, the morphological portrayal indicated the process of the total tumor lesion of the organ. The high proliferative activity of carcinoma cells, confirmed by the presence of many figures of pathological mitosis in almost all fields of view, was
maintained by the necessary conditions of their environment, namely, blood impregnation of the kidney parenchyma. This created a metabolic field with a high level of free-radical processes that promoted the tumor progression.

On the presented rat kidney preparations, significant blood filling of the vessels and hemorrhages in the parenchyma and connective tissue membrane were observed. The release of tumor cells forming strands or separate fields with a dense arrangement of proliferating cells completely veiled the renal structures and demonstrated the total growth of Guerin’s carcinoma during the development of diabetes in the body.

Morphological examination of the peritoneum

An aggressive growth of Guerin’s carcinoma transplanted under the skin in rats with induced severe comorbid DM was characterized by the full loss of tissue protective barriers and free transport of malignant cells not only to the parenchyma of various somatic organs, but also to the peritoneum, as a single connective tissue protective system of the abdominal cavity.

Figure 6 shows extensive areas of peritoneal blood supply in the close proximity to penetrating tumor cells. Along with multiple disparate areas of Guerin’s carcinoma cells, the formation of a strand-type structure was observed (see Figure 7 herein), which aggregated into large conglomerates and filled the entire peritoneal space due to actively proliferating pools of malignant cells (see Figure 8 herein).

Thus, the morphological examination of the metastatic lesions of organs (the ovary, the kidney, the peritoneum) in the female rats during the modeling of the growth of Guerin’s carcinoma on the background of diabetes mellitus made it possible to confirm the exclusively aggressive nature of the tumor behavior outside the primary tumor node and come closer to understanding the mechanisms of endogenous interaction between two different pathological processes within a single organism. And there, apparently, the dominant starting role is assigned to the biochemical

Figure 5. Fragments of the kidney in female rats in modeling of the growth of Guerin’s carcinoma on the background of diabetes mellitus. Hemorrhages and dense growth of tumor cells (the top row of photos), total metastatic lesion of kidney tissue (the bottom row of photos). Stained with hematoxylin-eosin, magnified x100, x20.
environment modulated by DM, in which the molecular mechanisms of metabolic regulation are disturbed.

Although the biological mechanisms of the DM modulation are not fully understood, studies have confirmed that the insulin/insulin-like growth factor (IGF) axis, hyperglycemia, inflammatory cytokines, and sex hormones create favorable conditions for the proliferation and metastasis of cancer cells. The insulin/IGF axis activates several metabolic and mitogenic signaling pathways. Hyperglycemia provides energy for the growth of cancer cells. Inflammatory cytokines affect the apoptosis of cancer cells [10]. Tumors often
require hyperactivity of the IGF system as a means of progressing the neoplastic process. Over-expression of autocrine/paracrine IGF by tumor cells or supporting stromal cells serves to stimulate cancer progression [13,14].

Thus, drawing structural and functional parallels between the two simulated processes, DM and the progression of Guerin's carcinoma, one can trace the relationship of certain events at the molecular and organ levels, associated with gender characteristics. Based on the data of research works on biochemical studies [8], in female rats of the main group, during the formation of a tumor node, the perifocal zone of the tumor, which is supersaturated with glucose, is characterized by high metabolic activity, but at the same time, the content of insulin-like growth factor 1 (IGF-I) is significantly lower, than that found in the tumors. On the contrary, in the tumor node itself, even with a relatively small volume, a highly aggressive environment is formed, combining moderate glucose saturation with an excess of IGF-I, which, like “gunpowder in a barrel”, explodes its protective armor and stimulates metastatic progression in a foreign territory, namely, the organs with abundant vascularization: the ovary, the kidney and the peritoneum.

In contrast, in the male rats, DM reduces glucose levels as well as IGF and IGFBP-2 in the carcinoma tumor itself and increases the IGF and IGFBP-2 levels without changing glucose concentrations in the perifocal zone. Since IGFBP-2 is significantly expressed in rapidly dividing cells characterized by high mobility, this could induce proliferation and growth progression only of the primary tumor node, but not metastasizing to other organs, which was confirmed by tumor volume indicators significantly exceeding those with standard growth.

**Conclusion**

Thus, the obtained morphological data, firstly, indicate the identity of the progression of the tumor lesions of various organs in diabetes and confirm not only the synergy of both pathological processes, but also the powerful pro-oncogenic effect of the comorbid state in diabetes in the implementation of the tumor growth program. Secondly, the emerging gender characteristics of the tumor progression, which, on the one hand, follows the path of active metastasizing under small primary tumors in female rats and, on the other hand, stimulates the growth of the primary focus without metastasis in male rats, correlates with varying degrees of tumor saturation and perifocal zone with glucose, and is also determined by the state of the insulin/insulin-like growth factor (IGF) axis. Of course, this dependence is determined by the hormonal profile, the role of which must be carefully studied.

**Statement on ethical issues**

Research involving people and/or animals is in full compliance with current national and international ethical standards.

**Conflict of interest**

None declared.

**Author contributions**

The authors read the ICMJE criteria for authorship and approved the final manuscript.
References


Influence of diabetes mellitus on free radical processes in the heart in rats with Guerin’s carcinoma and characteristics of malignant lesions depending on the gender of animals


National Medical Research Centre for Oncology, Rostov-on-Don, Russia, 344037, Rostov-on-Don, 14 liniya, 63, building 8

* Corresponding author:
+79281757704
iagor17@mail.ru

Abstract

**Aims.** To study the intensity of lipid peroxidation (LPO) and the activity of the main antioxidant protection enzyme: superoxide dismutase (SOD) in heart tissues and tumors in rats of both genders with Guerin’s carcinoma (GC) and the tumor growth against the background of diabetes mellitus (DM).

**Materials and methods.** Our research work was carried out in 80 outbread albino male and female rats, divided into 4 groups, with 10 animals of each gender in a group. The animals of two groups, namely, an intact animal group and a group of rats treated with alloxan DM (with a 5-fold increase in glucose levels) were subcutaneously transplanted with the Guerin’s carcinoma (GC) strain cells, and at the same time we used one group of the intact rats and another group of the animals with DM as the references. The content of malondialdehyde (MDA), diene conjugates (DC), and the SOD activity in the heart and the tumor tissues were determined by conventional spectrophotometric methods.

**Results.** The most pronounced changes were found in the heart in the female rats with isolated GC and GC growing against the background of DM: a more than threefold increase in MDA, with a significant increase in DC and a multiple increase in the SOD activity as compared with the intact animals. In the GC tissue, the dependence of the severity of the increase in the MDA content on the size of the tumor was traced: the maximum increase in both parameters was observed in males with GC tumor growing against the background of DM. In the males, the volume of the subcutaneous tumor nodes was 1.8 times greater than that in the reference group and in the females with combined pathology, while in the females with combined pathology the volume of their tumors was 1.3 times less than that in the reference group, although the area of tumor lesions in them was maximum due to extensive metastasizing.

**Conclusion.** DM has changed the specifics of oncogenesis depending on the gender of the animals. The identified gender differences in the redox status of the heart and the tumor in rats with combined pathology contribute to specifics of oncogenesis in males and females and determines their life expectancy.

Keywords
Heart, Free radical oxidation, Superoxide dismutase, Diabetes, Guerin’s carcinoma, Male and female rats

Imprint

Introduction
Cancer and diabetes are comorbid pathologies [1, 2]. Diabetes mellitus (DM) causes multiple organ dysfunction and aggravates the course of malignant pathology [3] as well as heart diseases including cardiovascular dysfunction [4]. It has been shown that in DM, the risk of developing heart failure is higher both in males and females [4].

The basis of cellular homeostasis is the balance of redox processes. The role of oxidative stress in the development and the course of both malignant pathology [5–8] and DM [9–12] is beyond doubt.

The evidence data available in the reference literature on the dynamics of changes in the activity of antioxidant enzymes during the development of DM in...
blood of patients and tissues of experimental animals are inconsistent [10], and in case of a tumor growth against the background of diabetes, comprehensive experimental studies of the oxidative status of different tissues have not been carried out.

Both in malignant pathology and in DM, the dependence of the course of some pathological processes and the metabolic change features on the gender of experimental animals has been identified. Thus, certain significant gender differences were found in the regulation of the plasminogen system and the timing of the formation of experimental melanoma in mice against the background of chronic neurogenic pain [13], the functioning of the hypothalamic-pituitary-adrenal axes in the body in male and female mice with transplanted melanoma [14], and the effect of knockout of the urokinase gene on the growth of melanoma in the experiment [15]. Only in males with DM, a decrease in insulin-like growth factors and the IGFBP-1 carrier protein was observed in the heart tissue [16]. A rat model of gestational DM has shown the presence of the gender differences in the regulation of lipid metabolism in the fetal liver [17].

Taking into account the role of free radical processes and the dependence of the course of the pathological processes on the gender of experimental animals, as well as the pronounced effect of DM on the development of heart diseases, the aim of our work was to study the intensity of lipid peroxidation (LPO) and indicators of the anti-oxidant protection in the heart of rats of both genders with DM, Guerin's carcinoma (GC) and their combined pathology.

Materials and methods

The study included outbread albino rats of both genders weighing 180-220 g originated from the stock kept by the “Research Center of Biomedical Technologies” (Branch Andreevka, Moscow Region). All manipulations with the animals were in full compliance with Directive 86/609/EEC on the Protection of Animals Used for Experimental and Other Scientific Purpose. The record of the experimental study was approved by the Commission on Bioethics at the National Medical Research Center for Oncology of the Ministry of Health of Russia, Record No. 21/99 by the Ethics Committee dated September 1, 2020.

The animals were divided into 4 groups (10 animals of each gender per group): 1) intact animals; 2) animals with DM; 3) animals with growth of transplanted Guerin’s carcinoma cells; 4) animals with growth of transplanted Guerin’s carcinoma cells against the background of DM. To reproduce experimental diabetes, alloxan was intraperitoneally injected at a single dose of 150 mg/kg of body weight, and blood glucose levels were measured during a week. At the time of tumor transplantation in the animals of the fourth group, the average blood glucose level was 25.4±1.2 mmol/l, while in the group of intact animals it was 5.2±0.3 mmol/l. The rats of the third and fourth groups after 1 week of persistent hyperglycemia were subcutaneously injected with 0.5 ml of a suspension of the Guerin's tumor cells in a 1:5 dilution with saline solution. 3 days after the injection of the suspension of Guerin’s carcinoma strain cells, the subcutaneous tumor growth was recorded. The animals of all groups were slaughtered with a guillotine 10 days after tumor transplantation in group 3 and 4.

The intensity of lipid peroxidation (LPO) processes was evaluated by the tissue contents of primary products - diene conjugates (DC) and TBA-positive products in terms of malondialdehyde (MDA) as the most stable secondary product of LPO. The activity of superoxide dismutase (SOD) was determined by the degree of inhibition of the reduction of nitroblue tetrazolium in the presence of the superoxide radical produced from the reaction of reduction of molecular oxygen by adrenaline in an alkaline medium; the enzyme activity was expressed in U/g of tissue. All indicators were determined in the 10% tissue homogenates by conventional spectrophotometric methods [18].

Statistical processing of the results was carried out with STATISTICA 10.0 using the Student’s t-test for two independent samples, as well as using the non-parametric Mann-Whitney test. The compliance of the samples with the normal distribution was assessed using the Shapiro-Wilk test. In the tables, the data are presented as the mean value ± standard error of the mean (M±m), the median and the values of the lower and upper quartiles are also indicated: Me (Q25; Q75). Differences were considered statistically significant at p<0.05 and tending towards statistical significance at 0.05<p<0.1.

Results

The content of LPO products: MDA and DC, as well as the activity of SOD in the heart of the rats are presented in Table 1 herein.
In the intact animals, there were no gender differences in the content of lipid peroxidation products in the heart reported. In the males, the MDA level remained unchanged both during the development of diabetes mellitus and the growth of Guerin’s carcinoma and in case of comorbidity, i.e. the malignant growth in diabetic rats. In contrast to the males, the females showed a sharp increase in the content of MDA in the heart tissue under all conditions studied. In diabetes mellitus, the level of MDA increased by a factor of 3, and with the growth of Guerin’s carcinoma, both against the background of diabetes and without the latter, it was found to be 3.2 times greater as compared with the intact animals. At the same time, a highly significant excess of the MDA content in the females against the males was observed: in diabetes mellitus it was 2.9 times greater, in case of Guerin’s carcinoma it was 3.2 times greater, and under comorbidity it was 3.6 times higher (p = 0.000000 in all cases).

The content of DC in DM in the heart of the males did not change significantly, and in the females it turned out to be reduced by 27.3% (p = 0.0005) as against the intact animals that resulted in the appearance of the gender differences: in the males, the level of this LPO product became higher than that in the females by 38.8% (p=0.0002). With Guerin’s carcinoma, an increase in the DC content was observed in the animals of both genders: in the males by 104% (p=0.000000), and in the females by 75.8% (p=0.0005) versus the intact animals of the corresponding gender. At the same time, with Guerin’s carcinoma, the DC level was higher than that found in the animals with DM: in the males by 71.6% and in the females by 141.7% (p≤0.00002). With the growth of Guerin’s carcinoma against the background of DM, the content of DC was lower than that in the animals with the malignant growth only: in the males by 43.5% (p=0.000000) and in the females by 22% (p=0.05). In this case, the level of the above mentioned product in the females remained higher by 37.2% than that recorded in the intact females (p=0.0001) and exceeded the level in the males.

### Table 1

The content of malondialdehyde, diene conjugates and the activity of superoxide dismutase in the heart tissue of rats with diabetes mellitus during the development of Guerin’s carcinoma

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Intact animals</th>
<th>Diabetes mellitus</th>
<th>Guerin’s carcinoma</th>
<th>Diabetes mellitus + Guerin’s carcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MDA, nmol/g</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>males</strong></td>
<td>16.03±1.96</td>
<td>14.87±2.66</td>
<td>14.86±1.2</td>
<td>12.86±1.13</td>
</tr>
<tr>
<td></td>
<td>17.37(13.59;19.86)</td>
<td>13.4(6.81;20.43)</td>
<td>14.86 (12.72;17.01)</td>
<td>12.48(9.04;16.14)</td>
</tr>
<tr>
<td><strong>females</strong></td>
<td>14.46±1.3</td>
<td>43.24±2.56</td>
<td>46.89±2.23</td>
<td>46.88±3.09</td>
</tr>
<tr>
<td></td>
<td>14.66(11.19;17.17)</td>
<td>43.03(38.71;46.29)</td>
<td>47.39(41.67;51.91)</td>
<td>45.11(39.23;56.27)</td>
</tr>
<tr>
<td></td>
<td>p=0.000000</td>
<td>p=0.000000</td>
<td>p=0.000000</td>
<td>p=0.000000</td>
</tr>
<tr>
<td><strong>DC, μmol/g</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>males</strong></td>
<td>20.61±2.17</td>
<td>24.5±1.71</td>
<td>42.05±1.5</td>
<td>23.75±1.52</td>
</tr>
<tr>
<td></td>
<td>21.62(14.87;25.94)</td>
<td>24.49(19.34;27.56)</td>
<td>42.05(37.94;46.16)</td>
<td>22.92(19.15;26.44)</td>
</tr>
<tr>
<td><strong>females</strong></td>
<td>20.69±0.81</td>
<td>15.05±1.06</td>
<td>36.37±3.62</td>
<td>28.38±1.39</td>
</tr>
<tr>
<td></td>
<td>21.07(18.41;21.6)</td>
<td>15.22(12.31;16.42)</td>
<td>34.21(28.38;45.19)</td>
<td>28.5(25.39;31.14)</td>
</tr>
<tr>
<td></td>
<td>p=0.000515</td>
<td>p=0.000000</td>
<td>p=0.000505</td>
<td>p=0.000000</td>
</tr>
<tr>
<td></td>
<td>p=0.000206</td>
<td></td>
<td>p=0.000023</td>
<td></td>
</tr>
<tr>
<td><strong>SOD, U/g</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>males</strong></td>
<td>30.29±3.43</td>
<td>14.02±1.58</td>
<td>8.95±0.82</td>
<td>8.69±0.93</td>
</tr>
<tr>
<td></td>
<td>31.72(28.29;37.25)</td>
<td>13.07(10.18;18.29)</td>
<td>8.85(7.61;11.09)</td>
<td>8.46(6.17;11.66)</td>
</tr>
<tr>
<td></td>
<td>p=0.000419</td>
<td>p=0.000010</td>
<td>p=0.010572</td>
<td>p=0.000010</td>
</tr>
<tr>
<td><strong>females</strong></td>
<td>8.38±0.82</td>
<td>8.71±1.33</td>
<td>46.17±10.86</td>
<td>52.88±4.6</td>
</tr>
<tr>
<td></td>
<td>8.38(7.45;9.68)</td>
<td>10.71(8.19;12.66)</td>
<td>47.6(13.81;71.5)</td>
<td>58.08(37.82;64.8)</td>
</tr>
<tr>
<td></td>
<td>p=0.00007</td>
<td></td>
<td>p=0.002739</td>
<td>p=0.000000</td>
</tr>
<tr>
<td></td>
<td>p=0.004540</td>
<td></td>
<td>p=0.003078</td>
<td></td>
</tr>
<tr>
<td><strong>SOD, U/g</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>females</strong></td>
<td>10.71±1.33</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>10.71(8.19;12.66)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>p=0.000000</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Notes:** Statistical significance of the differences: p - compared with the group of intact animals, p1 - compared with the group of animals with diabetes mellitus, p2 - compared with the group of animals with Guerin’s carcinoma, p3 - between males and females.
with combined pathology by 19.5% (p=0.037). In the males with combined pathology, no significant differences from the intact animals were observed.

Particularly pronounced differences between the males and the females were observed in the activity of SOD as the main antioxidant enzyme, which largely determined the severity of oxidative stress and also labially responded to the level of the LPO intermediates. In the males, there was a decrease in the SOD activity under all the studied pathological condition: the most pronounced reduction by 70.5% under the malignant growth of Guerin’s carcinoma only and by 71.3% with the tumor growth against the background of DM (p = 0.00001), while as in DM itself, the SOD activity was lower by 53.7% than in the intact males (p=0.0004) and higher than in both groups with Guerin’s carcinoma by 56.6-61.3% (p=0.01). In the females, in which the SOD activity in the heart in the intact state was 3.6 times lower than that found in the males (p = 0.000007), with the growth of Guerin’s carcinoma, the SOD activation was observed on average by a factor of 5.5-6. With the development of the tumor in the animals with DM, an increase in the enzyme activity was recorded to be 6.3 times versus the intact females and 4.8 times against the animals with DM (p=0.000000 in both cases).

An increase in the content of MDA was also demonstrated in the mitochondrial fraction of the heart in the female rats with DM by a factor of 1.9 and in those with the growth of Guerin’s carcinoma against the background of DM by a factor of 1.7 [19,20].

Having identified the significant gender differences in the severity of the changes in lipid peroxidation products and the SOD activity in the heart during the growth of Guerin’s carcinoma against the background of DM and in the animals without DM, taking into account the importance of free radical processes in a tumor invasion, we compared the data obtained with the characteristics of the tumor growth in males and females in the presence and absence of DM in the animals.

As can be seen from Table 2 herein, in the males with DM, the average tumor volume was by 75.4% (p=0.0041) larger than that found in the animals with the growth of Guerin’s carcinoma in the males without DM, while in the females, on the contrary, there was a tendency to inhibition of the tumor growth. In this case, in the females with DM, the average tumor volume was 3 times (p=0.0001) less than that recorded in the males. A distinctive feature of the tumor process developing against the background of DM in the females was a combination of small sizes of subcutaneous primary tumor nodes with a generalization of the process, accompanied by metastatic lesions of the parietal and visceral peritoneum, ovaries, kidneys and the development of ascites.

The study of the LPO parameters in the tumor tissue revealed some differences between the males and females. The content of MDA in the tissue of Guerin’s carcinoma, which developed against the background of DM, was 86.7% (p=0.0025) higher in the males than in the tumor of the animals without DM, and

<table>
<thead>
<tr>
<th>Parameters</th>
<th>MALES</th>
<th>FEMALES</th>
</tr>
</thead>
<tbody>
<tr>
<td>V tumor, cm³</td>
<td>68.14±8.38</td>
<td>119.5±12.4</td>
</tr>
<tr>
<td>MDA, nmol/g</td>
<td>0.722±0.076</td>
<td>1.348±0.162</td>
</tr>
<tr>
<td>DC, μmol/g</td>
<td>33.34±5.65</td>
<td>24.27±1.54</td>
</tr>
<tr>
<td>SOD, U/g</td>
<td>5.64±0.745</td>
<td>9.59±1.717</td>
</tr>
</tbody>
</table>

Table 2
The content of malondialdehyde, diene conjugates and the activity of superoxide dismutase in tumor tissue of rats with diabetes mellitus during the development of Guerin’s carcinoma

Notes: Statistical significance of differences: p - compared with the group of animals with Guerin’s carcinoma, p₁ - between males and females.
in the females there were no significant differences between these groups. As to the content of DC, only a tendency to a lower level (by 13.4%, p=0.074) was revealed with the tumor growth against the background of DM in the females. At the same time, in the tumor tissue of the males, the level of MDA and DC was higher than that found in the females, with the tumor growth in the animals with DM, the differences in MDA reached 3.5 times (p = 0.00005), and for DC the difference was 23.1% (p=0.029). This indicates a higher intensity of lipid peroxidation in the tumor tissue of the males compared with that in the females, especially in comorbidities. The SOD activity in the tumor of the males with DM was increased by 70% (p=0.049), and no significant differences therein were found in the tumor of the females (see Table 2 herein).

Discussion

Our comparative analysis of the content of the lipid peroxidation products in the heart showed that in DM the changes were detected only in the females and consisted of a 3-fold increase in MDA and a decrease in DC by 27%. In both groups with Guerin’s carcinoma, we observed in the females an accumulation of both LPO products and a multiple increase in the SOD activity in most animals, while the malignant growth in the males did not lead to an increase in the content of MDA, and the level of DC was increased only under the independent growth of Guerin’s carcinoma that was accompanied by a decrease in the SOD activity by a factor of 3.4-3.5 as compared with the intact males. Thus, as opposed to the males, the females were characterized by a sharp intensification of lipid peroxidation in the heart, which, under the conditions of the multiply increased SOD activity, indicates the development of pronounced oxidative stress in this most important organ.

In this case, the males with DM are characterized by a highly significant threefold excess of the tumor volume compared with the females. It can be assumed that one of the factors that ensure the greatest tumor growth in the males with DM is the intensification of lipid peroxidation and the activation of SOD in the tumor tissue, which protects tumor cells from the development of oxidative stress. The dependence of the tumor growth on the oxidative status has been also shown in earlier studies conducted by our Research Center [21-24]. This mechanism of the tumor progression is discussed by the latest reviews published in the reference papers abroad [25,26]. While in the females, generalization of the malignant process became possible due to a pronounced intensification of free radical processes in the cardiovascular system, and, consequently, damage to the vascular wall facilitating the extravasation of circulating tumor cells from the bloodstream into the tissues. It is the hematogenous route of metastasizing that is most likely appears in case of tumor cell dissemination to the kidneys, ovaries, and peritoneum, which is characteristic of the female rats with the development of a tumor process against the background of DM. Oxidative damage to the heart in the females with the malignant growth against the background of DM can contribute to a decrease in life expectancy, the average duration of which in case of Guerin’s carcinoma in the females with DM was 1.6 times shorter than that recorded in the females without DM.

Conclusions

1. Intensification of free radical processes in the heart in the female rats during the growth of Guerin’s sarcoma against the background of DM may reflect or rather contribute to the deterioration of the health state in the animals, leading to a more pronounced tumor invasion in them and resulting in their accelerated death.

2. Intensification of lipid peroxidation in the tumor itself, which is typical only for the males with DM, is one of the factors for increasing the size of the primary tumor, which has been found 3 times larger in the males than that recorded in the females with DM.

Statement on ethical issues

Research involving people and/or animals is in full compliance with current national and international ethical standards.

Conflict of interest

None declared.

Author contributions

The authors read the ICMJE criteria for authorship and approved the final manuscript.
References


Modifying effect of obesity on the content of sex hormones and their receptors in endometrial adenocarcinoma and its surrounding tissue

Oleg I. Kit, Elena M. Frantsiyants, Valeria A. Bandovkina*, Tatiana I. Moiseenko, Natalia V. Chernikova, Meri L. Adamyan, Yuriy A. Poryvaev, Natalia D. Cheryarina, Sergey V. Tumanyan, Svetlana V. Kornienko

National Medical Research Centre for Oncology, Rostov-on-Don, Russia, 344037, Rostov-on-Don, 14 liniya, 63, building 8
* Corresponding author: valerryana@yandex.ru

Abstract
Aims. To study the effect of comorbid pathology: obesity of degree 2-3 on the level of sex steroid hormones and their receptors in the tumor and its surrounding tissue in patients with endometrial cancer (EC).

Materials and methods. In 30 patients with endometrioid adenocarcinoma T1-3N0-1M0 (the main group, 15 females with obesity grade 2-3 (BMI ≥ 35); the reference group 15 females with normal BMI) in samples of the tumor and its perifocal zone taken after surgical treatment, the levels of estradiol (E2), estrone (E1), testosterone (T), progesterone (P4), androgen receptors (AR), progesterone receptors (RP4), estrogen receptors (ERα and ERβ) were determined by ELISA method. Statistical analysis was performed with STATISTICA 10.0.

Results. Obese EC patients showed longer healing of postoperative wounds, slow recovery, and more frequent tumor metastasizing to regional lymph nodes. In the tumor samples in all patients, compared with the intact endometrium, the levels of estrogens, testosterone and their receptors were higher. Obesity accompanying the malignant process led to a local increase in the levels of estrogens, testosterone, progesterone and AR, ERα and ERβ in the tumor. In the tumor samples, there were no significant differences from the presence of obesity in the levels of RP4. In the perifocal zone of the tumor in patients with comorbid pathology, compared with the parameters in the reference group, the level of E2, P4 and T was also higher, but the content of all steroid receptors was lower.

Conclusion. Obesity aggravates hyperestrogenism and progesterone deficiency in adenocarcinoma and increases its enrichment with the androgen and estrogen receptors with the prevalence of ERα over ERβ that may cause the autocrine-paracrine regulation of the growth and metastasizing of the malignant process in patients with endometrial cancer.

Keywords
Endometrial cancer, Obesity, Estrogens, Progesterone, Testosterone, Steroid hormone receptors

Imprint

Introduction
Endometrial cancer (EC) is a widespread heterogeneous disease, the progression of which is due to some genetic factors, comorbidities and environmental factors [1,2,3].

According to epidemiological studies, obesity may play an important role in the development of gynecological diseases, especially in uterine corpus cancer (UCC) [4,5]. Thus, with a rise in the body mass index (BMI) per each 5 kg/m², an increase in the risk of endometrial malignant tumors by 60% is noted [6]. Females with BMI ≥30 kg/m² have a 3-fold increased risk of UCC compared with the non-obese females (BMI <25), reaching an 8-fold risk in females with BMI ≥40. Obesity is most often associated with endometrioid carcinomas and may also increase the risk of non-endometrioid tumors [7].

Adipose tissue is now fully recognized as a metabolically active endocrine organ that secretes sex steroids, including estrogens, as well as adiponectin, visfatin, resistin, leptin, and tumor necrosis factor-α (TNFa) [5,8,9]. It is assumed that obesity affects the tissues of the uterus through its hormonal activity, pro-inflammatory effect and hyperinsulinemia [10]. However, the relationship between obesity and cancer of the reproductive organs remains largely a controversial issue due to the complexity of epidemiological studies to identify the actual causal relations [1].
Adipose tissue expresses aromatase, an enzyme, which catalyzes the endogenous conversion of androgen to estrogen. Thus, with the growth of the adipose tissue, the estrogen levels increase. Similarly, the amount of the sex-hormone binding globulin decreases hence the level of the bioactive estrogen in the circulating bloodstream elevates even more [9]. In addition, obesity is associated with diabetes, metabolic syndrome, and a pro-inflammatory state, which may contribute to endometrial carcinogenesis through an increased exposure to growth factors and other non-estrogenic mechanisms [11]. Taking into account the pandemic of metabolic and endocrine disorders in the modern world that indicates that a human being lives in a chronic stressful environment, characterized by the consumption of high-energy food combined with low physical activity [12], the study of the impact of obesity as comorbid pathology on the malignant process is topical.

The aim of our research work was to study the effect of the presence of comorbid pathology: obesity of degree 2-3 on the level of sex steroid hormones and their receptors in the tumor and its surrounding tissue in patients with endometrial cancer.

Materials and methods

Examined were 30 patients with endometrioid adenocarcinoma T1-3N0-1M0, mean age 64±3.2 years, including the main group with 15 females with obesity grade 2-3 (BMI≥35) and the reference group covering 15 females with the normal BMI values. Conditionally intact endometrium was obtained after surgical treatment of the patients with uterine myoma, who had the normal BMI values. All patients gave their written informed consent to conduct our scientific research. In the samples of the intact endometrium, the tumor and the perifocal zone of the latter, obtained after surgical treatment of the patients, the levels of estradiol (E2), estrone (E1), testosterone (T), progesterone (P4) (Cusabio, China), androgen receptors (AR), progesterone receptors (RP4), estrogen receptors (ERα and ERβ) (Cloud-Clone Corp. China) were detected by ELISA method. Our statistical analysis was performed with STATISTICA 10.0. The normality of distribution was assessed by the Shapiro-Wilk test, and the significance of differences between the groups was identified by the Kruskal-Wallis method.

Results

In the patients with endometrial cancer in the main group, where the malignant process developed against the background of obesity, more frequent tumor metastasizing to regional lymph nodes, slow healing of postoperative wounds and long recovery were found, compared with the patients in the reference group with the normal BMI values.

It was revealed that in the tumor samples collected from the patients both in the main and the reference group, compared with the parameters in the intact endometrium (Table 1), the following levels were higher: the level of E1 was 3.3 times higher and 10.7 times higher, respectively; E2 greater by a factor of 1.5 and 3.6; T greater by a factor of 1.4 and 3.2, respectively; RA higher by a factor of 2.3 and 4.7, respectively; ERα greater by a factor of 3.2 and 6.1 respectively; ERβ greater by a factor of 1.3 and 3.2, respectively. At the same time, we found that only in the tumors of the patients of the main group the level of P4 was 1.6 times higher than that in the intact endometrium. Significant differences in the content of RP4 in the tumor samples in the patients of the main and reference groups were not identified.

Our comparative analysis of the perifocal zones showed that in the patients of the main group, the level of E2 was 1.4 times higher, P4 5.7 times higher, T 4.3 times greater, but the content of all receptors was lower: RP4 1.9 times lower, AR 1.7 times smaller, ERα 1.9 times and ERβ 1.9 times lower, respectively.

In the tumor tissue of the patients with EC, the levels of E1 and E2 exceeded those in the samples of the perifocal zones: in the main group by a factor of 15 and 3.3, and in the reference group by a factor of 6.2 and 2, respectively. The content of testosterone in the main group was 1.5 times higher in the perifocal zone compared with the tumor region, while in the reference group, on the contrary, it was 1.3 times higher in the tumor than it was detected in the corresponding perifocal zone.

It is noteworthy that in the patients of the main group in the tumor and the perifocal zone, the level of progesterone did not have significant differences, exceeding the levels found in the intact endometrium, while in the reference group, on the contrary, the level of P4 in the perifocal zone was 3.9 times lower than that recorded in the tumor and, at the same time, did not exceed the values in the intact endometrium in any sample. In addition, the levels of AR, ERα and ERβ in the main group were higher in the tumor by a
factor of 2.5, 1.9 and 1.4, respectively, compared with its perifocal zone, while in the reference group, on the contrary, the content of AR, ERα and ERβ in the perifocal zone exceeded those in the tumor by a factor of 1.4, 1.9 and 3.2, respectively.

An analysis of the ratios in the patients of the main and the reference groups (see Table 2 herein) showed that hyperestrogenism was revealed in the tumor samples, compared with the intact endometrium, due to the prevalence not of estradiol, but estrone, and, so E1/T was 3.3 times and 2.4 times higher, respectively, while E2/T had no significant differences.

In addition, a progesterone deficiency was detected in the tumor samples, compared with the intact endometrium: E1/P4 and E2/P4 in the main group were 6.5 times higher and 2.2 times higher, respectively, and the same was applicable to the reference group: E1/P4 and E2/P4 were greater by a factor of 3.2 and by a factor of 1.4, correspondingly. It should be noted that against the background of the prevalence of estrone in the tumor samples, both in the reference and main group, the ratio of the estrogen receptors changed, with the prevalence of the alpha form: REα / REβ were 2.4 times and 1.9 times higher, respectively, compared with the intact endometrium.

In the perifocal zone of the tumor in the patients only of the reference group, an imbalance between estrogen and progesterone towards the prevalence of estrogens was revealed, compared with the intact endometrium: E1 / P4 and E2 / P4 were 2.4 times and 2.8 times higher, while in the perifocal zone of adenocarcinoma in the patients of the main group, E1/P4 and E2/P4 were 2.1 and 1.4 times lower than those in the intact endometrium, respectively. The balance between es-

<table>
<thead>
<tr>
<th>Indicators</th>
<th>Normal endometrium</th>
<th>Reference group</th>
<th>Main group</th>
<th>Perifocal zone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estrone ng/g tissue</td>
<td>96.6±8.64</td>
<td>320.1±14.1</td>
<td>1029±50.64</td>
<td>68.58±2.59</td>
</tr>
<tr>
<td>Estradiol ng/g tissue</td>
<td>563.44±27.3</td>
<td>847.75±32.89</td>
<td>242.7±13.87</td>
<td>604.6±37.38</td>
</tr>
<tr>
<td>Progesterone ng/g tissue</td>
<td>0.86±0.05</td>
<td>0.9±0.087</td>
<td>0.23±0.017</td>
<td>1.3±0.11</td>
</tr>
<tr>
<td>Testosterone ng/g tissue</td>
<td>1.0±0.058</td>
<td>1.4±0.068</td>
<td>1.1±0.071</td>
<td>4.75±0.27</td>
</tr>
<tr>
<td>RP4 ng/g tissue</td>
<td>8.66±0.17</td>
<td>8.54±0.39</td>
<td>34.52±1.23</td>
<td>18.39±0.97</td>
</tr>
<tr>
<td>AR ng/g tissue</td>
<td>1.05±0.06</td>
<td>2.4±0.198</td>
<td>3.37±0.16</td>
<td>1.95±0.12</td>
</tr>
<tr>
<td>ERα ng/g tissue</td>
<td>0.65±0.022</td>
<td>2.1±0.225</td>
<td>3.93±0.13</td>
<td>2.03±0.11</td>
</tr>
<tr>
<td>ERβ ng/g tissue</td>
<td>1.04±0.059</td>
<td>1.40±0.11</td>
<td>4.48±0.26</td>
<td>2.31±0.17</td>
</tr>
</tbody>
</table>

Notes: significant differences compared with: 1 – intact endometrium; 2 – indicators in similar samples of the reference group; 3 – indicators in the perifocal zone.
trogens and androgens in the perifocal zones of the tumor in the patients with EC was shifted towards androgens: E1/T and E2/T were 6.7 and 4.4 times lower than those in the intact endometrium in the main group and 2.1 and 1.5 times smaller in the reference one. Similar to the case with the tumor tissue, the alpha form of the estrogen receptors prevailed in the perifocal zone compared with the intact endometrium: REα/REβ was 3 times higher in the main group and 1.8 times higher in the reference group.

**Discussion**

To properly explain the carcinogenesis of endometrial cancer, the “indisputable estrogen hypothesis” has conventionally been used, according to which a progestin deficiency against the background of an excessive amount of estrogens stimulates proliferation and suppresses endometrial apoptosis [13]. The endometrial cells, in response to sex steroid hormones, primarily estrogens and progesterone, multiply, differentiate and regress. These hormonal-induced physiological changes require complex paracrine interactions between certain endometrial cell types. When the hormonal balance between estrogens, progestins, and androgens is disrupted, the endometrium may become neoplastic that may lead to cancer [14].

In recent years, there has been an increase in the number of studies providing evidence of changes in tissue-specific concentrations of steroids and their receptors, which does not necessarily coincide with those in blood. At the same time, steroid metabolism in peripheral tissues is considered to be the key way in which, in particular, the endometrium can respond to local physiological demands and “fine tune” either activation or inhibition of processes dependent on the steroid hormone receptors [15]. The ability of malignant tumors to independently synthesize and metabolize hormones, against the background of the presence of their receptors, allows a neoplasm to autonomously regulate its growth [16, 17, 18].

Our study showed that, indeed, the tumor samples contained an increased amount of estrogens, with estrone prevailing over estradiol. The pathology accompanying the malignant growth in the form of obesity elevated the level of estrogens in the tumor without changing the dominant role of E1 over E2. This can be explained by the fact that adipose tissue is an endocrine organ capable of producing a large amount of steroid hormones, including estrogen [19]. In addition, in our study, it was found that against the background of obesity in the patients of the main group, the content of progesterone increased both in the tu-

---

### Table 2

Ratios of sex steroid hormones and their receptors in patients depending on the presence of obesity in tumor samples and perifocal zone

<table>
<thead>
<tr>
<th>Indicators (c.u.)</th>
<th>Normal endometrium</th>
<th>Reference group</th>
<th>Main group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>tumor</td>
<td>tumor p/zone</td>
<td>tumor</td>
</tr>
<tr>
<td>E1/T</td>
<td>96.6±4.2</td>
<td>228.6±12.3</td>
<td>46.8±3.1</td>
</tr>
<tr>
<td></td>
<td>P₁=0.000410</td>
<td>P₁=0.0000910</td>
<td>P₁=0.000412</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>P₂=0.000380</td>
</tr>
<tr>
<td>E2/T</td>
<td>563.4±23.4</td>
<td>605.6±36.2</td>
<td>386.1±22.7</td>
</tr>
<tr>
<td></td>
<td>P₁=0.000412</td>
<td>P₁=0.0000912</td>
<td>P₁=0.000410</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>P₂=0.000380</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>P₃=0.000412</td>
</tr>
<tr>
<td>E1/P4</td>
<td>112.3±5.4</td>
<td>355.6±21.1</td>
<td>223.6±15.4</td>
</tr>
<tr>
<td></td>
<td>P₁=0.000410</td>
<td>P₁=0.0000912</td>
<td>P₁=0.000380</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>P₂=0.0000970</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>P₃=0.000412</td>
</tr>
<tr>
<td>E2/P4</td>
<td>665±32.7</td>
<td>942±35.0</td>
<td>1846.5±120.3</td>
</tr>
<tr>
<td></td>
<td>P₁=0.000380</td>
<td>P₁=0.000412</td>
<td>P₁=0.0000412</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>P₂=0.0000412</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>P₃=0.000412</td>
</tr>
<tr>
<td>REα/REβ</td>
<td>0.63±0.04</td>
<td>1.5±0.09</td>
<td>1.11±0.08</td>
</tr>
<tr>
<td></td>
<td>P₁=0.000410</td>
<td>P₁=0.0000970</td>
<td>P₁=0.000410</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>P₂=0.0000380</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>P₃=0.000412</td>
</tr>
</tbody>
</table>

Notes: significant differences compared with: 1 – intact endometrium; 2 – indicators in similar samples of the reference group; 3 – indicators in the perifocal zone.
mor and in the perifocal zone. However, the calculation of the ratios of estrogens to progesterone showed an undoubted prevalence of estrogens. A shift in the balance between estrogen and progesterone towards a more pronounced hyperestrogenic state is known to increase the risk of endometrial cancer, and obesity induces anovulation, which reduces progesterone protection of the endometrium from high levels of unrestricted endogenous estrogen. This leads to a constant exposure of the endometrium to high levels of estrogen, which produces a mitogenic effect on the endometrial tissue, stimulating the growth and reproduction of endometrial glands and stromal cells [20].

It is known that the hormone therapy for first-line endometrial cancer consists of progestin treatment, the effectiveness of which is only 25% [21]. Our studies show that only in patients with obesity, the level of P4 increases in the tumor and its perifocal zone, however, in the tumor samples, a rise in the level of progesterone receptors was not detected, the growing content of which was found only in the perifocal zone, reaching its maximum in the patients with the normal BMI values. It is possible that it is just the peculiarity that is responsible for the ineffectiveness of the progestin therapy found in some patients with EC.

There is evidence that obesity contributes to an increase in androgen synthesis, a phenomenon often observed in polycystic ovarian disease that is another risk factor for endometrial cancer [22]. Therefore, it can be assumed that an excess of androgens can also have a transforming effect on the endometrial cells [14].

At the same time, the controversial role of androgens in the endometrium is reported, which can demonstrate both their pro- and anti-proliferative effects [23]. Thus, in individuals with a sex change from a woman to a man, long-term use of testosterone contributes to uterine atrophy and thinning of the endometrium, which indicates the anti-proliferative and apoptotic effects [24]. However, in mice subjected to ovariectomy, data have been obtained indicating that in the absence of ovarian hormones (estrogen and progesterone), the use of dihydrotestosterone promotes the proliferation of endometrial cells [25].

In our study, the patients with EC of the main group showed a significant increase in their testosterone levels, both in the tumor and in the perifocal zone, compared with those in patients without comorbid pathology. The calculation of the ratios of estrogen to androgens showed that, despite the increased level of testosterone, in the tumor samples, the balance was shifted towards the prevalence of estrone. In addition, one should take into account the fact that androgens can act as a prohormone that increases the effects of estrogen, especially in postmenopausal and obese women, while the endometrium contains a sufficient amount of enzymes necessary for the synthesis of estrogens [19, 26].

The expression of steroid hormone receptors, as well as their etiological and prognostic role in endometrial cancer, has been the subject of extensive research in the current and past decades. The use of hormonal therapy in the treatment of endometrial cancer, targeting both RP4 and ER, demonstrates a low efficiency of such treatment [26].

In our study, it was found that both the tumor and its perifocal zone are characterized by a high level of estrogen and androgen receptors, compared with the intact endometrium, however, an increase in the content of progesterone receptors has been found only in the perifocal zone of adenocarcinoma. The presence of comorbid pathology, obesity, has made its own adjustments to the receptor status of the studied tissues, in the tumors in the patients of the main group, the level of AR and ER has been recorded to be higher than that in the perifocal zone, while in the reference group, it is the perifocal zone considered as the tissue more enriched with receptors. In a 2016 study by Kamal A.M. et al., it has been reported that in EC metastasizing, an increase in the AR expression is detected [27]. In addition, it is believed that the steroid hormone receptors are capable of co-regulation. Thus, REs bind to specific DNA sequences called estrogen response elements on the RP4 promoter and thus are able to increase their expression, while P4, on the other hand, can suppress the expression both of REs and its own ones. At the same time, testosterone can suppress the expression of the RP4 gene, and the maximum expression of AR in the endometrium can be detected during the follicular phase [28].

Another interesting feature is the change in the ratio of estrogen receptors towards the prevalence of ERs. In the human endometrium, ERα and ERβ exhibit cell-specific expression patterns during the menstrual cycle, ERα is present in the epithelial cells lining the glands and the lumen during the proliferative phase, at a time when the levels of circulating estrogens are rapidly elevated due to the growth of antral follicles containing cells aromatase-expressing granulose, but
decreased during the secretory phase, whereas ERβ does not reflect a dynamic change in expression in the stromal or epithelial cells and is present in endothelial cells and multiple immune cell populations that are ERα-negative [15]. The prevalence of the α-form of estrogen receptors may demonstrate the proliferative potential of a malignant tumor.

**Conclusion**

Obesity as a disease against the background of which endometrial cancer develops, has an essential effect on the hormonal receptor background in the tumor and its surrounding area, aggravating hyperestrogenism and progesterone deficiency in adenocarcinoma and increasing its enrichment with androgen and estrogen receptors, with a predominance of ERα over ERβ, which can cause the autocrine-paracrine regulation of growth and metastasizing of the malignant process.

**Statement on ethical issues**

Research involving people and/or animals is in full compliance with current national and international ethical standards.

**Conflict of interest**

None declared.

**Author contributions**

The authors read the ICMJE criteria for authorship and approved the final manuscript.

**References**

22. Comim FV, Hardy K, Franks S. Adiponectin and its receptors in the ovary: further evidence for a link between obesity and hyperandrogenism in polycystic ovary syndrome. PLoS One 2013;8:e80416
Development of an experimental model of tumor growth under hypothyroidism


National Medical Research Centre for Oncology, Rostov-on-Don, Russia, 344037, Rostov-on-Don, 14 liniya, 63, building 8

* Corresponding author: neskubina.irina@mail.ru

Abstract

Aim. Our aim has been to develop an experimental model of the tumor growth against the background of hypothyroidism in rats of both genders in order to study possible influence made by hypothyroidism on progression of malignant tumors of various histological types.

Materials and methods. In our studies we have used 100 out-bred albino rats of both genders, with an individual body mass of 150-180 g. The female rats (n=30) and the male rats (n=30) have received Mercazolil at a day dosage of 2,5 mg/100g of the body weight for 30 days. After hypothyroidism in the treated rodents had been confirmed, one group of them (15 females and 15 males) were subcutaneously inoculated with the Guerin's carcinoma cells, and another group (covering other 15 females and other 15 males) has been undergone to transplantation of the Sarcoma 45 cells. The reference group has included the rats of the 3 day period, we have estimated with a radioisotope analysis (RIA) standard assay kits (Immunotech, Czech Republic) the levels of the thyroid hormones in blood of the tested animals as follows: Triiodothyronine (T3) (pM/L), total Thyroxine (T4) (pM/L) and Thyroid-Stimulating Hormon (TSH) (μIU/mL). The obtained data have been processed with Statistica 10.0.

Results. Upon the treatment with Mercazolil, we have found in the females a decrease by a factor of 7,3 in the total level of Thyroxine and an increase by a factor 1,6 in the TSH level (p<0,05), while in the males we have recorded a reduction by a factor of 2 in the total level of Thyroxine and an increase by a factor of 1,5 in the TSH level (p<0,05). In this case, the average sizes of the tumors in the female rats with Guerin's carcinoma and hypothyroidism have been found smaller than those found in the reference group as given below: upon expiration of 4 days they are 1,3 times smaller (p<0,05), upon expiration of 7 and 10 days the volumes have been found 1,4 times smaller (p<0,05); upon expiration of 14 days the volumes have been recorded to be 1,5 times less (p<0,05), upon expiration of 18 days they have been reported to be 1,3 times less (p<0,05), and upon expiration of 21 days they have been estimated to be 1,4 times less (p<0,05). As to the males with Guerin's carcinoma and hypothyroidism, the average sizes of their tumors as against the reference group data have been recorded to be smaller as follows: upon expiration of 4 days they are found 13,3 times less; upon expiration of 7 days they have been recorded to be 7,5 times smaller; upon expiration of 10 days the volumes have been estimated to be 1,9 times less (p<0,05), and upon expiration of 14 days they have been found to be 2,6 times less. The survival rate in the female rats in the main test has been recorded to be 1,6 times higher (p<0,05) against the data in the reference group, while the survival rate in the males has not shown any significant differences therein.

In the female rates with S 45 growing against the background of hypothyroidism the average sizes of the tumors have been found to be less than those identified in the reference group as follows: after 4 days, the sizes have been recorded to be 1,4 times less (p<0,05); after 7 and 10 days they have been recorded to be 1,6 and 3,2 times smaller, respectively (p<0,05); after 14 days they have been found to be 3,9 times less, and after 18 days they have been recorded to be 4,8 times less. In the males at tumor growth stage 1, the tumor sizes have increased 3,1 times as against 4 days of the tumor growth; upon expiration of 10 days the sizes have been found to be 7,1 times greater as compared with the previous period; upon expiration of 2 weeks they have increased 1,5 times (p<0,05); upon expiration of 18 and 21 days the tumor sizes have been recorded to be greater by a factor of 2,3 and by a factor of 1,6, respectively (p<0,05). The life spans in the female rodents in the main test group has been reported to be longer by a factor of 1,8 (p<0,05) than it has been the case with the reference group, and the average life span in the males has reached 21 days.

Conclusion. We have revealed that in the female rates diagnosed with hypothyroidism the sizes of the subcutaneous tumor nodes of Guerin's carcinoma and S 45 show slower progression as against that recorded in the reference group, and

Issue 21. February 2022 | Cardiometry | 41
the life span recorded in the above rodents has been found as significantly longer, while in the male rats with hypothyroidism we have observed an irregular, slower progression of the tumor nodes of Guerin’s carcinoma and S 45 within the period of 14 days, but subsequently we have detected the same progression rate as it is the case with the reference group data.

**Keywords**
Hypothyroidism, Experimental model, Rats, Guerin’s carcinoma, Thyroxine, Triiodothyronine, Thyroid-Stimulating Hormon

**Imprint**

**Introduction**

The dynamic capability of biological systems to maintain the required flexible homeostatic balance by responding to environmental challenges is a salient feature of the normal health state in an organism. In this context, hormones released by the thyroid gland (thyroid hormones referred to as TH) are double-play actors in the homestatic regulation: first they are participants therein, and second they are at the same time controllable units. They are targeted at a wide range of metabolism effects, however, to a greater extent, at the same time they are externally controllable agents. Dysfunction of the thyroid gland including hypothyroidism is found in females more often as compared with males: the incidence rate is reported to be 2-9 times higher [1], but unfortunately in addition to the fertility and bone tissue aspects there is no proper understanding of what is possible influence made by the gender aspects on the typical features of various diseases: that's applicable to international level research and refers both to clinical studies and epidemiologic cohorts. At present, the generally accepted experimental approaches with the use of mice as models to investigate actions and effects produced by thyroid hormones have involved the experimental use of mice of the same gender only; moreover sometimes it is even ignored which gender is subjected to research studies. In 2014 Nature has appealed to the scientific community to make stronger efforts in exploring specific influences made by the gender on manifestation, prediction of possible outcome and treatment of diseases that subsequently has been supported by the Endocrine Society [2]. Besides “American thyroid association guide to investigating thyroid hormone economy and action in rodent and cell models” published in 2013 has issued recommendations to conduct gender-specific studies, since responding to TH disorders may differ depending on the gender [3].

Based on the mouse model, H. Rakov et al. have detected some apparent gender-related differences in the functional behavior, metabolic and biochemical parameters in mice diagnosed with hypothyroidism [4]. The above researchers have shown that it is precisely the gender that is an important modifier of actions and effects of the thyroid hormones that result in different phenotype-, metabolism- and biochemistry-related markers of hypothyroidism in male with female mice.

Experimental evidence data have confirmed the fact that Thyroxine (T4) and Triiodothyronine (T3) make their proliferative and anti-apoptotic action and effects on cancer cells by regulating gene expression and stimulating estrogen-like effects that indicates that there is a relationship between the dysfunction of the thyroid gland and the risk of the onset of cancer [5].

However the relevant epidemiologic studies have supplied us with some contradictory data [6]. In a meta-analysis of some observation data published before 2019 there has not been revealed any statistically significant relation between hypothyroidism and a risk of the onset of breast cancer, but at the same time two recent research studies have declared that there is a lowering of risk of the onset of breast cancer linked with hypothyroidism [7, 8]. In contrast, some other investigations [9] have demonstrated that there is a higher risk of breast cancer in females with hyperthyreosis as compared with females having no dysfunction of the thyroid gland that has been supported by the results from another meta-analysis and a randomized study [7]. Following this way, revealed has been a more aggressive impact of the brain metastases under primary lung, breast and kidney cancer as well as skin melanoma on the hormonal status in the affected patients.
The authors thereof are of the opinion that the para-
neoplastic abnormalities in the content of the thyroid
hormones and cortisol should be taken into consider-
ation in treatment of this sort of patients.

In view of the existing clinical context, when not
only women, but also men suffer from hypothyroid-
ism, taking into account the fact that the consequences
of this disease, among them cancer, may be gender-de-
pendent, it is reasonable to apply an experimental
model in mice to provide an extensive, a more com-
prehensive, investigation of a possible influence of the
gender on the actions and effects made by the thyroid
hormones.

Development of experimental models is an in-
tegral part of research to discover the nature of ma-
lignant tumors and search for new ways to produce
the desired effect thereon [10, 11, 12, 13, 14]. In this
connection, the models confirming the substantial
contribution of comorbidity to the onset and pro-
gression of malignant tumors are of particular inter-
est. So, METHOD OF THE MELANOMA B 16 MA-
lIGNANT GROWTH IN MICE MODIFICATION WITH
CHRONIC PAIN (Pat. RU No. 2650587 С1),
METHOD OF STIMULATING CHRONIC PAIN OF
MALIGNANT GROWTH IN LUNG RATS (Pat. RU
No. 2676641 С) and METHOD FOR REVERSING
GENETICALLY DETERMINED INHIBITION OF
GROWTH OF A MALIGNANT TUMOR IN EXPER-
IMENT (Pat. No.2718671 С1) [15,16,17] have shown
that the chronic neurogenic pain is a factor stimulat-
ing the growth and progression of malignant tumors of
different histogenesis, and it is even capable of re-
versing the genetically determined inhibition of the
melanoma growth in animals with the urokinase gene
knockout that implies an inhibition of its growth.

The aim of our experimental research has been a
development of an experimental model of a tumor
growth against the background of hypothyroidism in
rats of both genders in order to investigate an influ-
ence made by hypothyroidism on progression of ma-
lignant tumors of different histological structures.

Materials and methods

Our experiment has been conducted using 100 out-
bred albino rats of both genders, with an individual
body mass of 150–180 g. The experimental rodents
have been supplied by the Federal State Medical & Bi-
ological Institution “Research Center of Biomedical
Technologies” (Branch Andreevka, Moscow Region)
at the Federal Medical & Biological Agency. The labor-
atory animals were kept under natural lighting condi-
tions with free access to water and food. The research
in the animals was completed in full compliance with
the Directive 86/609/EEC on the Protection of Ani-
mals Used for Experimental and Other Scientific Pur-
pose, in accordance with the International Guiding
Principles for Biomedical Research Involving Animals
and Order No. 267 “Approval of the Rules of Laborato-
ry Practice” dated June, 19, 2003 issued by the Minis-
try of Health of the Russian Federation. Manipulations
with animals were performed in the box in compli-
ance with the generally accepted rules of asepsis and
antisepsis.

We have used in our research cell lines of Guerin’s
carcinoma and Sarcoma 45 delivered by the Federal
State Budgetary Institution “N. N. Blokhin Russian
National Oncology Research Center” at the Ministry
of Health of the Russian Federation. The material for
the inoculation was harvested from donor rats on tu-
mor development day 12–16. The above mentioned
laboratory animals were subcutaneously inoculated
with the Guerin’s carcinoma and Sarcoma 45 (S 45)
cells by the standard subcutaneous injection of a vol-
ume of 0,5 ml of the tumor suspension cells in saline
solution with a dilution ratio 1:10 in the right scapular
region.

The outbred albino female rats (n=30) and male
rats (n=30) have received Mercazolil at a day dose
of 2,5 mg/100 g of body mass (the total dose is
75 mg/100 g of body mass) for 30 days. No avoidance
of eating has been noted in the experiment with the
animals; they have gained in their individual weight,
but at the same time recorded have been deterioration
of their external skin and hair coat appearance, some
mobility problems and sleepiness in them. Upon ob-
taining the state of stable hypothyroidism with the use
of Mercazolil for the purpose of investigations of an
impact of the disease on the growth of malignant tu-
mors of different histological structures, the animals
of both genders (30 males and 30 females) with their
confirmed hypothyroidism diagnosis have been divid-
ed into two test groups: one test group (15 females and
15 males) has been inoculated subcutaneously with
the Guerin’s carcinoma cells, and another test group
(15 females and 15 males) has been inoculated sub-
cutaneously with the Sarcoma 45 cells. For the pur-
purpose of tracing the hypothyroidism-free progression
of the malignant tumor in the animals, we have used
the reference cohort of the rats of both genders, who had reached their sexual maturity and who had not be subjected to hyperthyroidism reproduction, to cover one group (n=10 females and n=10 males) inoculated subcutaneously with the Guerin’s carcinoma cells and another (n=10 females and n=10 males) transplanted with the Sarcoma 45 line cells, at the same dose and with the same volume of the cell lines as it is the case with the main test group. Upon expiration of one month, within the 3 day period, we have estimated with a radioisotope analysis standard assay kits (Immunotech, Czech Republic) the levels of Triiodothyronine (T3) (pM/L), total Thyroxine (T4) (pM/L) and Thyroid-Stimulating Hormon (TSH) (μU/mL).

The obtained experimental data have been processed with Statistica 10.0. The data have been analyzed for their compliance with the normal distribution law using the Shapiro-Wilk test (for small sample sizes). To make the comparison of more than two independent groups (for independent samples) we have utilized the Kruskal-Wallis test (when an independent variable consists of two or more categorical, independent groups). The table data are presented in the M±m form, where M is the arithmetic mean, and m is the standard error of the mean; in this case, p<0.05 has been taken as the level of statistical significance. The obtained results have been statistically processed in accordance with general guidelines applicable to medical research studies.

Results

Upon treatment of the female rats with Mercazolil for 30 days per os, we have found in their blood a decrease in the total level of Thyroxine by a factor of 7,3 and an increase in the TSH level by a factor of 1,6 (p<0,05), while in the males, who have been administered with Mercazolil for 30 days, a reduction 2 times in the total level of Thyroxine has been recorded and an increase 1,5 times in the TSH level has been reported (p<0,05) (see Table 1 herein). The detected low levels of Thyroxine and high levels of TSH after 30-day administration of Mercazolil at a dose of 2,5 mg/100 g of body weight bear witness to the fact that the designed stable state of hypothyroidism has been achieved.

Some specific features of the tumor growth upon the standard Guerin’s carcinoma inoculation and those upon the same type inoculation against the background of hypothyroidism in rats of both genders are presented in Tables 2 and 3 herein.

### Table 1

<table>
<thead>
<tr>
<th>Groups of animals</th>
<th>Hormones</th>
<th>T4 (pM/L)</th>
<th>T3 (pM/L)</th>
<th>TSH (μU/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact females (n=10)</td>
<td></td>
<td>61,2±5,9</td>
<td>1,05±0,1</td>
<td>0,085±0,007</td>
</tr>
<tr>
<td>Mercazolil-treated females (n=30)</td>
<td></td>
<td>8,41±0,81</td>
<td>1,25±0,11</td>
<td>0,14±0,013</td>
</tr>
<tr>
<td>Intact males (n=10)</td>
<td></td>
<td>75,58±7,2</td>
<td>1,46±0,12</td>
<td>0,08±0,06</td>
</tr>
<tr>
<td>Mercazolil-treated males (n=30)</td>
<td></td>
<td>38,06±3,4</td>
<td>1,11±0,09</td>
<td>0,12±0,009</td>
</tr>
</tbody>
</table>

Note: ¹ significant differences as compared with the respective values in the intact animals of the same gender p<0,05.

The subcutaneous tumor (Guerin’s carcinoma) in the females in the main test group (n=15) has become detectable 4 days after the inoculation, and its average volume has reached at that time 0,125 cm³. Upon the inoculation, the above tumor has been produced in 80% of the females, while in 20% (n=3) of the female rats with hypothyroidism no Guerin’s carcinoma has developed. At week 1 stage of the tumor progression we have recorded an increase 18,2 times in the tumor mass as against the mass recorded on tumor growth day 4; on 10 day we have found an increase 5,8 times in the volume compared with the previous stage, and upon expiration of 2 weeks the volume has been recorded to be greater by a factor of 2,1. 3 weeks after the Guerin’s carcinoma inoculation we have observed an inhibition of the tumor growth, so that the volumes of the tumor in the females in the main test group have been found to be 1,4 times (p<0,05) greater than those recorded after 18 days. Since day 11, with the tumor node enlargement in the animals, we have revealed some alopecia spots on the skin surface, which have shown a tendency towards their growth.

Upon expiration of 24 days of the tumor growth in the females in the main test group reported has been the first death case. Their average life span has been recorded to reach 29 days, with its maximum of 33 days (see Table 2 herein).

As to the females rats in the reference group (n=10), with the growing Guerin’s carcinoma tumor against the normal levels of the thyroid hormones and TSH, the subcutaneous tumor was detectable after 4 days; its average volume has reached 0,165 cm³; the tumor cell productivity has achieved 100%. One week after the inoculation the average volume of the tumor
has increased by a factor of 1.9 (p<0.05) as compared with the data recorded after 4 days of the growth; the tumor volume increase by a factor of 5.8 has been identified upon expiration of 10 days, and upon expiration of 2 weeks it has been recorded to be increased 2.4 times as compared with the previous stages. Upon expiration of 18 days of the experiment the average volume of the tumor has been reported to be 1.3 times greater (p<0.05), and 3 weeks after the inoculation it has become 1.9 greater (p<0.05) as against the previous measuring stages. Beginning with week two, in the female rats in the reference group detected have been tumor necrosis cases. The first death case in the reference group has been reported on day 13, and the last case thereof has been recorded on day 26 of the experiment; the average life span of the animals has reached 18 days.

It has been revealed that in the females with the Guerin's carcinoma tumor growing against the background of hypothyroidism the average volumes recorded at all measuring stages are smaller than those found in the reference group as indicated below: on 4 day smaller by a factor of 1.3 (p<0.05), on day 7 and 10 it has decreased by a factor of 1.4 (p<0.05); upon expiration of 14 days it has been found 1.5 times smaller (p<0.05); upon expiration of 18 days it has been recorded 1.3 times smaller (p<0.05), and on day 21 it has become 1.4 times smaller (p<0.05). In this case the survival of the female rats in the main test group has been found to be 1.6 times higher (p<0.05) than it is the case with the rats in the reference group. The first death case in the main test group has been reported on day 24, i.e. 14 days later than it is the case with the animals in the reference group.

Our survey of the data dynamics of the tumor growth of inoculated Guerin’s carcinoma against the background of hypothyroidism is presented in Table 3 given herein.

The subcutaneous tumor (Guerin’s carcinoma) in the male rats in the main test group (n=15) has become palpable 4 days after the inoculation, and its average volume has reached at that time 0.0375 cm³. The capacity of the inoculated tumor cells to grow has been found in 100% of the males. At week 1 stage of the tumor growth, the tumor volume has increased by a factor of 13.3 as compared with the growth day 4 volume; after 10 days the volume has become larger by a factor of 15.9 against the data obtained in the previous test period; upon expiration of 2 weeks the volume has been recorded to be greater by a factor of 1.96 (p<0.05), and upon expiration of 18 days it has been detected to

### Table 2

<table>
<thead>
<tr>
<th>Time of testing</th>
<th>Main test group Hypothyroidism + Guerin’s carcinoma (averaged tumor V cm³)</th>
<th>Reference group Guerin’s carcinoma only (tumor V cm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 days</td>
<td>0.125±0.012³</td>
<td>0.165±0.058</td>
</tr>
<tr>
<td>7 days</td>
<td>2.275±0.53³</td>
<td>3.18±0.33</td>
</tr>
<tr>
<td>10 days</td>
<td>13.21±9.26³</td>
<td>18.4±2.42</td>
</tr>
<tr>
<td>14 days</td>
<td>27.28±1.62³</td>
<td>44.76±3.98</td>
</tr>
<tr>
<td>18 days</td>
<td>55.94±5.4³</td>
<td>70.3±7.84</td>
</tr>
<tr>
<td>21 days</td>
<td>75.73±6.88³</td>
<td>107.96±9.01</td>
</tr>
<tr>
<td>Appearance of alopecia on skin surface</td>
<td>Beginning with day 11</td>
<td>Not available</td>
</tr>
<tr>
<td>Appearance of skin necrosis</td>
<td>Not available</td>
<td>Beginning with day 14</td>
</tr>
<tr>
<td>Average life span (days)</td>
<td>29.3±1.16³</td>
<td>18.2±1.38</td>
</tr>
<tr>
<td>The first death case recorded in the group</td>
<td>On day 24</td>
<td>On day 13</td>
</tr>
<tr>
<td>The last death case recorded in the group</td>
<td>On day 33</td>
<td>On day 26</td>
</tr>
</tbody>
</table>

Note: ³ significant differences as compared with the respective values in the animals of the reference group p<0.05.

### Table 3

<table>
<thead>
<tr>
<th>Time of testing</th>
<th>Main test group Hypothyroidism + Guerin’s carcinoma (tumor V cm³)</th>
<th>Reference group Guerin’s carcinoma only (tumor V cm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 days</td>
<td>0.0375±0.0037³</td>
<td>0.5±0.035</td>
</tr>
<tr>
<td>7 days</td>
<td>0.5±0.0023³</td>
<td>3.82±0.27</td>
</tr>
<tr>
<td>10 days</td>
<td>7.94±0.80³</td>
<td>14.74±1.15</td>
</tr>
<tr>
<td>14 days</td>
<td>15.61±1.395³</td>
<td>40.68±3.8</td>
</tr>
<tr>
<td>18 days</td>
<td>44.9±3.74³</td>
<td>52.84±5.48</td>
</tr>
<tr>
<td>21 days</td>
<td>72.93±7.09³</td>
<td>77.5±6.25</td>
</tr>
<tr>
<td>Appearance of skin necrosis</td>
<td>Not available</td>
<td>Beginning with day 7</td>
</tr>
<tr>
<td>Average life span (days)</td>
<td>23.7±2.12³</td>
<td>20.0±1.27</td>
</tr>
<tr>
<td>The first death case recorded in the group</td>
<td>On day 20</td>
<td>On day 14</td>
</tr>
<tr>
<td>The last death case recorded in the group</td>
<td>On day 25</td>
<td>On day 24</td>
</tr>
</tbody>
</table>

Note: ³ significant differences as compared with the respective values in the animals of the reference group p<0.05.

The subcutaneous tumor (Guerin’s carcinoma) in the male rats in the main test group (n=15) has become palpable 4 days after the inoculation, and its average volume has reached at that time 0.0375 cm³. The capacity of the inoculated tumor cells to grow has been found in 100% of the males. At week 1 stage of the tumor growth, the tumor volume has increased by a factor of 13.3 as compared with the growth day 4 volume; after 10 days the volume has become larger by a factor of 15.9 against the data obtained in the previous test period; upon expiration of 2 weeks the volume has been recorded to be greater by a factor of 1.96 (p<0.05), and upon expiration of 18 days it has been detected to
be larger by a factor of 2.9. Later, 3 weeks upon the inoculation, the tumor volumes in the males in the main test group have increased 1.6 times (p<0.05) as compared with the respective data on day 18. The first death case in the main test group has been recorded on day 20 of the experiment. The average life span has reached in this case 23.7 days with its maximum of 25 days (see Table 3 herein).

As to the male rats in the reference group (n=10), their subcutaneous tumor has become detectable on day 4 after the inoculation with its volume of 0.5 cm³; upon expiration of 7 days after the inoculation its average volume has become larger by a factor of 7.6; upon expiration of 10 days thereafter the tumor volume has increased 3.9 times and 14 days thereafter 2.8 times as against the measurements taken before. 18 days after the inoculation we have observed an inhibition of the tumor growth with an increase by a factor of 1.3 in its volume (p<0.05), and upon expiration of 21 days the volume has been reported to be larger by a factor of 1.5 (p<0.05). The first death case in the reference group has been recorded on day 14 of the Guerin’s carcinoma inoculation experiment, and the last death case has been observed therein on day 29, so that the average life span in this group has been reported to be 27 days.

Considering the male rats in the main test group with growing Guerin’s carcinoma against the background of hypothyroidism, as compared with the data obtained in the reference animals, the average volumes of the tumor at experiment stages from day 4 till day 14 have been found smaller as follows: on day 4 smaller by a factor of 13.3, on day 7 smaller by a factor of 7.5, on day 10 smaller by a factor of 1.9 (p<0.05), and on day 14 smaller by a factor of 2.6. However on day 18 and 21 we have not observed any significant differences therein from the data recorded in the males in the reference group.

Our survey of the experimental data obtained by us on the tumor volumes and life spans in the female rats with inoculated Sarcoma 45 against the background of hypothyroidism is given in Table 4 herein.

The subcutaneous tumor of S 45 in the main test group female rats (n=15) has become palpable on day 4 after the tumor cell transplantation, and its average volume has reached 0.29 cm³. The experimental tumor in the female rats has been produced in 100% of the inoculation cases. At the week 1 stage of the tumor growth, the volume of the tumor has become larger by a factor of 2.3 as against its 4 day growth; on day 10 we have observed an increase by a factor of 1.4 in the tumor volume (p<0.05), as compared with the sizes recorded before; upon expiration of 2 weeks the volume has become larger by a factor of 1.4 (p<0.05).

Upon expiration of 18 days after the S 45 inoculation, we have observed an inhibition of the tumor growth, and the recorded sizes thereof have shown no differences therein from those measured before. On day 24 of the experimental tumor growth period in the main test group females we have observed the first death case, and the average life span has been reported to be 32 days with its maximum of 33 days (see Table 4 herein).

As to the examined female rats in the reference group (n=10) with growing S 45 against the background of the normal values of thyroid hormones, the tumor has been palpable underneath the skin on day 4 with the tumor node average volume of 0.23 cm³, and in this group the tumor has been produced in 100% of the inoculation cases. Upon expiration of 1 week after the inoculation, the average volume of the tumor has increased 4.8 times as against the measurements taken on day 4; upon expiration of 10 days it has been recorded to be 2.8 times larger and after 2 weeks it has been observed to be 1.8 greater as compared with the respective previous experiment periods of time (p<0.05).

On day 18 of our experiment the average sizes of the growing tumor has been reported to be increased by a factor of 1.3 (p<0.05). The first case of the death

<table>
<thead>
<tr>
<th>Time of testing</th>
<th>Main test group Hypothyroidism + S 45 (tumor V cm³)</th>
<th>Reference group S 45 (tumor V cm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 days</td>
<td>0.29±0.012</td>
<td>0.23±0.020</td>
</tr>
<tr>
<td>7 days</td>
<td>0.68±0.06¹</td>
<td>1.1±0.10</td>
</tr>
<tr>
<td>10 days</td>
<td>0.95±0.09¹</td>
<td>3.08±0.31</td>
</tr>
<tr>
<td>14 days</td>
<td>1.39±0.14¹</td>
<td>5.42±0.50</td>
</tr>
<tr>
<td>18 days</td>
<td>1.4±0.15¹</td>
<td>6.68±0.7</td>
</tr>
<tr>
<td>Average life span (days)</td>
<td>32.4±1.2¹</td>
<td>18.0±1.38</td>
</tr>
<tr>
<td>The first death case recorded in the group</td>
<td>On day 24</td>
<td>On day 12</td>
</tr>
<tr>
<td>The last death case recorded in the group</td>
<td>On day 34</td>
<td>On day 20</td>
</tr>
</tbody>
</table>

Note: ¹ significant differences as compared with the respective values in the animals of the reference group p<0.05.
of the animal in the reference group has been recorded on day 12, and the last case of the death has been reported on day 20, so that the average life span has reached 18 days.

We have revealed that the average volumes of the tumor in the females with S 45 growing against the background of hypothyroidism, measured at all stages of the experimental tumor progression, are smaller as compared with those found in the reference group animals: on day 4 the volume has been recorded to be smaller by a factor of 1,4 (p<0,05), on day 7 and day 10 smaller by a factor of 1,6 (p<0,05) and by a factor of 3,2, respectively; upon expiration of 14 days of the experiment it has decreased 3,9 times, and after 18 days of the experiment a reduction by a factor of 4,8 has been observed.

In this case, the survival for the female rats in the main test group has been reported to be 1,8 higher (p<0,05) than it is the case with that in the reference group rats. The first death case in the main group with S 45 has been recorded 12 days later as compared with the respective data obtained in the reference group.

The dynamics of progression of the subcutaneous S 45 tumor against the background of hypothyroidism in the male rats and their average life span data are indicated in Table 5 herein.

Table 5
Tumor growth dynamics and survival data in male rats with inoculated Sarcoma 45 (S 45)

<table>
<thead>
<tr>
<th>Time of testing</th>
<th>Main test group Hypothyroidism + S 45 (tumor V cm³)</th>
<th>Reference group S 45 (tumor V cm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 days</td>
<td>0,125±0,018</td>
<td>0,25±0,020</td>
</tr>
<tr>
<td>7 days</td>
<td>0,39±0,18</td>
<td>0,56±0,08</td>
</tr>
<tr>
<td>10 days</td>
<td>2,75±0,40</td>
<td>3,36±0,58</td>
</tr>
<tr>
<td>14 days</td>
<td>4,05±0,60</td>
<td>4,2±0,7</td>
</tr>
<tr>
<td>18 days</td>
<td>9,44±0,99</td>
<td>9,24±0,7</td>
</tr>
<tr>
<td>21 days</td>
<td>14,8±1,5</td>
<td>11,8±1,5</td>
</tr>
<tr>
<td>Appearance of skin necrosis</td>
<td>Not available</td>
<td>Beginning with day 14</td>
</tr>
<tr>
<td>Average life span (days)</td>
<td>21,3±1,21</td>
<td>20,0±1,38</td>
</tr>
<tr>
<td>The first death case recorded in the group</td>
<td>On day 15</td>
<td>On day 14</td>
</tr>
<tr>
<td>The last death case recorded in the group</td>
<td>On day 22</td>
<td>On day 22</td>
</tr>
</tbody>
</table>

Note: 1 significant differences as compared with the respective values in the animals of the reference group p<0,05.

The S 45 tumor located subcutaneously in the male rats in the main test group (n=15) has become identifiable on day 4 after the experimental transplantation thereof, and its average volume has reached 0,125 cm³. The experimental tumor productivity has been produced in 100% of the inoculation cases in the males. At the week 1 stage the volume of the tumor has increased by a factor of 3,1 as against the measurements taken on day 4 of the experiment; on day 10 the volume thereof has become larger by a factor of 7,1 as compared with the respective previous stage; 2 weeks after the tumor transplantation the volume has been recorded to be greater by a factor of 1,5 (p<0,05); upon expiration of 18 days it has become larger by a factor of 2,3, and upon measuring on day 21 it has been found to be greater by a factor of 1,6 (p<0,05). The average life time has been recorded to be 21 days with its maximum of 22 days (see Table 5 herein). Considering the male rats in the reference group (n=10) with growing S 45 against the background of the normal values of thyroid hormones including TSH, the experimental subcutaneous tumor has become palpable on day 4 of the experiment, and the tumor node volume has reached 0,25 cm³; the experimental tumor inoculable capacity has achieved 100%. 1 week after the inoculation, the average volume of the experimental tumor has become 2,2 times larger as compared with that recorded on day 4; upon expiration of 10 days of the experiment it has been reported to be 6 times greater, and after 2 weeks the sizes thereof have been found to be larger by a factor of 1,25 (p<0,05) as against the data obtained in the respective previous period. On day 18 the average volume of the tumor has increased 2,2 times, and upon expiration of 3 weeks it has become greater by a factor of 1,3 (p<0,05). The first death case in this reference group has been observed on day 14, and the last death has been reported on day 22, so that the average life time in the animals has reached 20 days. As to the male rats in the main test group with growing S 45 against the background of hypothyroidism, it has been revealed that the average volumes measured within the period from day 4 till day 7 are smaller as listed below: on day 4 smaller by a factor of 2 and on day 7 by a factor of 1,4 (p<0,05), respectively. Upon analyzing the volume data related to test stages 10–21 days of growing S 45 no significant differences in the tumor volumes between the main test group males and the reference male rats have been detected. The average life spans in the males in the main test group have shown no differences from the values recorded in the males of the reference group.
Conclusion

Thus we may conclude that in the outbread albino female rats diagnosed with hypothyroidism (according to evidenced low values of total Thyroxine (T4) and high values of Thyroid-Stimulating Hormone (TSH) in serum) we have observed a delayed increase in the volumes of the tumor nodes produced by Guerin's carcinoma and S 45 upon their subcutaneous inoculation as compared with the respective progression tumor data observed in the reference group, and the life span has been recorded in them to be significantly longer. As to the outbread albino male rats with confirmed hypothyroidism (according to the confirmed low values of total Thyroxine (T4) and high values of Thyroid-Stimulating Hormone (TSH) in serum), we have found that the volumes of the tumor nodes, developed upon the subcutaneous Guerin's carcinoma and S 45 inoculation, have demonstrated an irregular pattern of their growth with some progression slow-down within the first 14 days of the experiment, followed by no-difference effects in the volume increase dynamics between the test and reference group. In this case, no significant differences in the life span values have been revealed. We think these effects should be attributed to a deficiency of Thyroxine as the main thyroid hormone responsible for many aspects in tumorogenesis. The pronounced effect recorded in the female rats in our study is determined by the predominance of estrogens as participants in the control and regulation in the thyroid gland performance.

Statement on ethical issues

Research involving people and/or animals is in full compliance with current national and international ethical standards.

Conflict of interest

None declared.

Author contributions

The authors read the ICMJE criteria for authorship and approved the final manuscript.

References

14. Zhukova GV, Shikhlyarova AI, Loginova LN, Protasova TP. Effects of combined exposure to low-intensity electromagnetic radiation of the millimeter range and


Decision support system in radiology for fast diagnostics of thoracic diseases under COVID-19 pandemic conditions

Ilya M. Borodyansky*

Southern Federal University, Russia, 347900, Taganrog, Shevchenko str, 2

*Corresponding author: ilay2002@inbox.ru

Abstract

In the present article the relevance of using DSS under the current conditions for image recognition and, as a more specific application, for the purpose of additional assistance rendered to medical experts (radiologists) in their decision-making and preparing findings upon assessment of X-ray images is considered. The paper analyzes the requirements for some expert DSS and their main characteristics that they should have; considered and selected is the necessary software for making rapid diagnoses of diseases of the thorax. All these modern requirements and characteristics are met by the Deep Learning Studio (DLS) software, which allows using deep convolutional neural network Inception V3 to teach this network and further obtain optimal results in the recognition and diagnosis of diseases of the thorax by assessing X-ray images.

As a result of this study, a ready-made DSS intended for use by medical institutions for additional assistance to radiologists to prepare findings according to X-ray images has been obtained.

Keywords

DSS, DLS, transfer learning, Inception V3 neural network, X-ray images, pneumonia, myocarditis

Imprint


At present, information technologies have been rapidly introducing into the broadest areas of human activity, and in this sense the impact made by digitalization in the spheres of society related to natural science, education, medical care, etc. is especially noticeable. We all have witnessed a huge push towards the growth of digitalization, “a global process that is subjugating the planet and even the space beyond its borders more and more every day” [1] during the global Covid-19 pandemic. The worldwide pandemic of coronavirus infection has revealed some deep-seated problems in society that have already been experienced by the society, but have been not so acute. For example, it is a well-known fact that there is a problem of providing primary care by qualified medical experts. Similar difficulties in finding narrow-field experts also affect the secondary link of municipal medical institutions (advisory centers, hospitals, etc.). The scarce specialties include, in particular, radiology.

The basic function of the lungs is to saturate the blood with oxygen, and during illness this function is significantly weakened. Therefore, the heart has to work in an extremely abnormal mode. Operating in this mode leads to rapid fatigue of the heart muscle.

The SARS-CoV-2 virus increases coagulation, resulting in the formation of blood clots. Damage to the walls of blood vessels can also provoke their inflammation and detachment of blood thrombi, which can lead to a heart attack, stroke or pulmonary embolism. Viral myocarditis (inflammation of the heart muscle fibers of an infectious nature) is also a very dangerous complication after the Covid-19 disease. The diagnosis of myocarditis is based on patient complaints and instrumental examinations, the most informative of which are CT and MRI of the heart, which are capable of showing pathological structural changes and the presence of an inflammatory process.

Recently, medical consultation systems based on the “second opinion” principle have found a widespread application: they allow properly assessing images and interpret the results [2].

An important task is to design medical decision support systems (DSS), which are information systems functioning autonomously or as a part of medical information systems (MIS). To reduce the severity of the problem, it is necessary to apply an approach to the development and implementation of the above information systems and DSS from the standpoint of the system analysis, one of the stages of which is
mathematical modeling, including a construction and an analysis of deterministic models [3]. The decision support system allows you to use the data obtained, on the basis of which it helps the doctor in decision-making and also provides information support for the decision being made [4–7].

Radiography is the most common tool for reviewing and further analysis of images, necessary for screening, diagnosis, and treatment of diseases, including pneumonia. However, it is estimated that two thirds of the world's population do not have access to radiological diagnostics. With the use of automated radiography systems at the expert level, this technology can also improve rendering of the medical care service and increase access to high-quality medical imaging expertise in those parts of the world where access to qualified radiologists is limited.

However, in order to implement full automation of the X-ray imaging in medicine, it is required to solve a set of complex tasks.

For example, it will be necessary to standardize the format and quality of the output of images produced by various X-ray equipment types. The absence of a standard regulating the parameters and quality of images will not guarantee the stability of the model and the reliability of the results upon data transfer from one device to another.

Next, you will need to add regular quality check of the model by submitting a reference test sample verified by doctors. At the same time, the quality of the model will be constantly tested. And finally, confidence thresholds should be built into the model, and, if exceeded, the images are to be submitted to the doctor for classification. Based on the current state of our primary and secondary health care, we are not yet ready to fully automate the process.

By DSS, we will understand an interactive automated system that helps the user (the decision maker (DM)) using data and models to identify and solve problems and make decisions. The DSS should be capable to work with interactive queries with a query language that is easy enough to learn.

According to D. Power [8, 9], the DSS has the following four main features:

1) DSS uses both data and models;
2) DSS is designed to help DM in decision-making for poorly structured and unstructured tasks;
3) DSS supports, but does not replace, decision-making by experts;
4) the purpose of using the DSS is to increase the efficiency in decision-making.

The typical features of an intelligent decision support system are the following:

– a clearly defined limitation of the subject area;
– availability of a knowledge base;
– separation of declarative and procedural knowledge (facts and decision-making mechanisms);
– capability to make decisions in unique problem situations for which the algorithm is not known in advance and is formed from the source data in the form of chains of decision-making rules from the knowledge base;
– capability to solve the problem under conditions of incompleteness, unreliability, ambiguity of the initial data and the lack of quantitative estimates of alternatives;
– capability to deduce decision rules well in time and answer specific user questions;
– using the interface that is most acceptable for the user of the given specialty [10].

There are some interesting features which an intelligent system may have and which are related to knowledge bases (KB), and one of them is such a property of machine learning as a modification of its own KB in the process of an intelligent system performance, an adaptation to a problem area. It is similar to a human ability to “gain experience”.

Machine Learning is an extensive subsection of artificial intelligence that studies methods for constructing algorithms capable of learning. There are two types of teaching: 1. Case-based learning, or inductive learning, is based on identifying patterns in empirical data. 2. Deductive learning involves the formalization of experts’ knowledge and their transfer to a computer in the form of a knowledge base. Deductive learning is usually attributed to the field of expert systems, so the terms machine learning and case-based learning can be considered synonymous [11, 12].

There is one area of AI that has achieved the greatest success in recent years, namely the field of image recognition and convolutional neural networks. In some tests, AI algorithms outperform humans in image recognition. Here are two examples: Large Scale Visual Recognition Challenge and German Traffic Sign Recognition Benchmark.

Therefore, our idea is to apply AI to the field of image recognition in the processes, where doctors are engaged, namely, to the analysis of X-ray images.
In the differential diagnosis of respiratory diseases, it is extremely important to identify the key radiological syndrome that allows the doctor to outline the potential list of diseases and apply additional examination methods [13–15].

The most informative method of the X-ray examination in respiratory diseases and diagnosis of viral pneumonia (including Covid-19) is a computed tomography (CT). This method is the most relevant under the current conditions [13–15].

To teach our neural network model, a pre-marked data set will be needed and, most importantly, the presence of a validation sample will also be a good advantage, however if it is not available, it will be possible to take a small part of the marked training sample, remove it from the set before teaching our model, and after training, use it as a validation one.

There are a lot of datasets containing X-ray images of the lungs, both of a healthy and pathological nature. After studying several examples of suitable datasets, our preference was given to a dataset freely hosted on Kaggle Chest X-Ray Images (Pneumonia), since it fits several parameters such as given below:

1) the dataset is already marked up;
2) large training sample size (5216 images);
3) availability of a validation sample;
4) it's an authoritative source.

All these parameters allow us evaluating this dataset as the most convenient to use.

The initial data for verification were taken from the Russian official website of State Budgetary Institution “Scientific Clinical Center for Diagnostics and Telemedical Technologies of the Department of Healthcare”, (https://tele-med.ai/), Moscow, and the Society of Radiologists, Moscow.

For our analysis of radiographs, all lung images were checked for image quality, and images of poor quality or poorly digitized were removed.

When trying to teach our model, we face three serious problems:

1) Insufficient data: as we have already said, it is difficult to find eligible datasets for each task, and it was a great luck for us to find them.

2) Lack of capacity: a system capacity is a big problem, it is almost impossible to teach a serious model at home; renting computers with powerful GPUs or virtual machine in the cloud is not always possible; the platforms with free access often have low capacities, and the huge disadvantage of renting a computer is the lack of knowledge necessary to configure this machine, when an extra worker is needed for this purpose.

3) Lack of time: much time is needed to teach the model.

Thus, we use the transfer training, which helps us retrain the final layer of the already trained Inception V3 model to new categories from scratch.

The main problem in training a neural network is a computing capacity, but in addition to that, besides the theoretical knowledge in the field of artificial intelligence, programming skills at a sufficiently high level may be required. Deep Learning Studio (DLS), on the other hand, simplifies the creating of architecture, training and retraining of the model, followed by the subsequent analysis of training outcomes as much as possible.

Since it was decided to use the Deep Learning Studio software in our research, it was also necessary to convert the data in the dataset into the form that the program could process [16]. For that aim the special Python3 program was developed, the task of which was to quickly and conveniently make a table, in which each image would contain a record to classify it. The program successfully produced such tables, and, after that, the archive with the tables and images was uploaded to DLS.

After uploading the data, the network training process was carried out several times, until it was possible to achieve a verification accuracy approximately of 74 percent (see Figure 1 herein).

With an increase in the number of the training epochs, the accuracy of the DSS will be significantly higher.

And the already trained network was considered as a DSS. We managed to process the X-ray images from the hospital, and the findings were obtained as indicated herein (see Figure 2).

Our DLS has a convenient interface, which allows you to upload images for processing via a tab in the browser, and, in our opinion, it greatly simplifies the work by a doctor in making a diagnosis. It makes the system user-friendly for applications by a medical institution, since every expert who has an access thereto can use one and the same trained network, and there is no need for deep knowledge in the field of AI, so that all this allows the doctor to better concentrate on making a diagnosis.
Statement on ethical issues

Research involving people and/or animals is in full compliance with current national and international ethical standards.

Conflict of interest

None declared.

Author contributions

The authors read the ICMJE criteria for authorship and approved the final manuscript.

References

1. Development of the digital economy: theoretical and practical significance for the agro-industrial com-
Gas exchange readjustments in response to hypoxia and hypercapnia exposure in Magadan region military service draftees

Inessa V. Averyanova*, Sergei I. Vdovenko

“Arktika” Scientific Research Center FEB RAS, 685000, Russia, Magadan, 24 Karl Marks St.

*Corresponding author:
Inessa1382@mail.ru

Abstract

Object. Our study identified gas exchange and external respiration characteristics during hypoxia and hypercapnia exposure in young men of the Magadan Region.

Materials and methods. A comprehensive survey in young men of military age, 18-21 yr., permanent residents of the Russia's Northeast, was conducted. A hypoxic-&-hypercapnic respiration test with no CO2 absorption was used. Before and after respiration, using indirect calorimetry method, we analyzed gas composition in exhaled air, external respiration variables, body energy expenditure at rest, respiratory quotient, ventilation equivalents for oxygen and carbon dioxide (Carbonic gas analyzer, Medgraphics VO2000 gas meter). Statistical data processing was performed with Statistica 7.0 package.

Results. Significant post-respiratory dynamics in most indicators of gas exchange and external respiration was found. In response to hypoxia and hypercapnia effects, an increase in the energy consumption at rest, in minute volume of body temperature and pressure saturation, in carbon dioxide emission and oxygen consumption per minute was observed with significantly decreased oxygen utilization factor.

Conclusion. Effects of hypoxic-&-hypercapnic test can be seen as pronounced readjustments in analyzed variables: intensified metabolism at the test peak and that reduced below baseline in the recovery period. In this case, breathing patterns are readjusted with pronounced increase in pulmonary ventilation and higher values in breathing depth in comparison with the baseline value at each stage of the recovery period, up to its 3rd minute.

Keywords
Young men, Gas exchange, Hypercapnia, Hypoxia, Rebreathing, Indirect calorimetry

Introduction

In scientific community, hypoxic and hypercapnic stimuli are supposed to reinforce each other in the regulation of physiological functions [1, 2, 3]. In fact, hypoxia with no hypercapnia does not cause breathing activation [2, 4, 5]. The respiratory system is mainly responsible for achieving and maintaining an appropriate arterial blood gas composition that is the tension of oxygen (Po2) and carbon dioxide (Pco2), thus to a certain extent, the hydrogen ion concentration [6].

Hypoxic and hypercapnic stimuli have an adaptive influence which intensifies the physical performance, and enhances the cardiovascular and respiratory functional capabilities [2]. Previous investigations on inhalation of mixture with higher amount of carbon dioxide proved the combined action of hypoxic-hypercapnic stimuli only to be able to maintain the proper internal environment of the body [7].

The average intensity of the hypercapnia and hypoxia effects, as well as their various combinations, has a compensatory importance in developing body adaptive responses since it improves the mechanisms of resistance to extreme factors [1]. Chemo-reflex demonstrates dominance in ventilation and cardiovascular autonomic regulation. Peripheral carotid chemoreceptors respond primarily to hypoxia [8, 9] while brain chemoreceptors respond primarily to hypercapnia [10].

Indirect calorimetry is widely used for measuring energy consumption, both at resting and functional tests that study energy homeostasis. This method most accurately assesses gas exchange responses and readjustments [11].

This study examined young men to investigate gas exchange responses under rebreathing test.

Materials and methods

Forty three males of military age, 18–21 yr., participated in the Magadan Region Military Commissariat
Training and Conscription Department Survey. The mean age was 20.0±0.3 yr. A rebreathing test with no CO2 absorption was used as a functional exercise. Subjective baseline exhaled O2 and CO2 values (%) were determined using the Carbonic gas analyzer. During the test, each subject made three deepest possible exhalations into the Douglas type bag. Further breathing was performed from that sealed bag for three minutes with the nose closed with a clip [12]. After the test completion, the gas mixture left in the bag was analyzed for the CO2 and O2 percentage amount.

We used the Medgraphics VO2000 metabolograph to analyze gas exchange and respiratory system parameters prior to the rebreathing test, at its peak, as well as at 2nd and 3rd minutes of the recovery. Energy consumption (Kcal, kcal/min; REE, kcal/day), their percentage to standard level (REE/Pred, %), respiratory volume (Vt BTRS, mL), respiration rate (RR, cycle/min), respiratory minute volume (VE BTRS, L/min), respiratory quotient (RQ, arb. units), oxygen consumption and carbon dioxide production rates (VO2, VCO2, mL/min) in ratio to respiration rate (VO2, VCO2, mL/RR), exhaled oxygen and carbon dioxide concentration (FETO2, FETCO2, %), oxygen and carbon dioxide ventilation equivalent (VE/VO2, VE/VCO2), and oxygen utilization factor (Ox. Util. Fact., mL/L) were calculated.

Results

Table 1 attached hereto shows data on indirect calorimetry at rest, after rebreathing, as well as at 2nd and 3rd minutes of the recovery period. We can observe significant dynamics through nearly all studied gas exchange indicators in the post-rebreathing period. The hypoxia and hypercapnia study showed statistically significant increase in Kcal, REE, REE/Pred, RR, VE BTRS, the CO2 production per minute (VCO2), the O2 consumption per minute (VO2), as well as correlation of those values with the respiratory rate, VE/VO2, and the oxygen concentration for each subject in the air they exhaled (FET O2) with a significantly lowered oxygen utilization factor.

The following subjective variables demonstrated a statistically significant decrease at the second minute of the recovery period as compared to those in

<table>
<thead>
<tr>
<th>Indicators</th>
<th>Stage of Test</th>
<th>Difference Significance Level</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Post Rebreathing</td>
</tr>
<tr>
<td>KCal (kcal/min)</td>
<td>1.37±0.04</td>
<td>1.93±0.13</td>
</tr>
<tr>
<td>REE (kcal/day)</td>
<td>2001±61.5</td>
<td>2875.9±189.7</td>
</tr>
<tr>
<td>RQ (arb. units)</td>
<td>0.78±0.02</td>
<td>1.00±0.03</td>
</tr>
<tr>
<td>REE/Pred (%)</td>
<td>111.5±2.9</td>
<td>161.4±11.3</td>
</tr>
<tr>
<td>RR (cycle/min)</td>
<td>16.5±0.7</td>
<td>16.7±0.8</td>
</tr>
<tr>
<td>Vt BTRS (mL)</td>
<td>575.4±22.2</td>
<td>882.6±62.4</td>
</tr>
<tr>
<td>VE BTRS (L/min)</td>
<td>8.6±0.2</td>
<td>14.3±0.9</td>
</tr>
<tr>
<td>VCO2 (mL/RR)</td>
<td>15.1±0.8</td>
<td>25.6±2.2</td>
</tr>
<tr>
<td>VCO2 (mL/min)</td>
<td>224.7±8.6</td>
<td>383.4±22.7</td>
</tr>
<tr>
<td>VO2 (mL/min)</td>
<td>289.4±8.9</td>
<td>398.9±27.9</td>
</tr>
<tr>
<td>VO2 (mL/RR)</td>
<td>19.4±0.9</td>
<td>26.3±2.3</td>
</tr>
<tr>
<td>VE/VO2 (arb. units)</td>
<td>39.4±1.0</td>
<td>37.6±1.0</td>
</tr>
<tr>
<td>VE/VCO2 (arb. units)</td>
<td>30.3±0.8</td>
<td>37.7±1.6</td>
</tr>
<tr>
<td>FET CO2 (%)</td>
<td>3.3±0.1</td>
<td>3.4±0.1</td>
</tr>
<tr>
<td>FET O2 (%)</td>
<td>16.7±0.1</td>
<td>17.2±0.1</td>
</tr>
<tr>
<td>Ox. Util. Fact. (arb. units)</td>
<td>34.1±0.9</td>
<td>28.8±1.3</td>
</tr>
</tbody>
</table>

Results were processed with Statistica 7.0. The Shapiro-Wilk test was used to check out distribution of measured variables for normality. Results of parametric processing methods were presented as the mean value and its error (M±m). Statistical significance for differences was determined with the Student’s t-criterion for dependent samples. The critical level of significance was taken to be equal to or less than 0.05.

Table 1
Indirect calorimetry in 18–21 year old males at rest and different stages of post rebreathe
the post-rebreathing period: Kcal, REE, REE/Pred, Vt ВТPS, VE ВТPS, VCO2 (mL/min), VO2 (mL/min), VCO2 (mL/RR), and VO2 (mL/RR) with no changes observed in RR, VE/VO2, and Ox. Util. Fact. At the third minute of the recovery, the values of Vt ВТPS, VE ВТPS, and VCO2 (mL/min) were significantly lower than those recorded at the second minute, but still higher than the resting values, which suggested an incomplete recuperation in comparison with the baseline. We could also observe an incomplete, vs. the baseline, recovery in RQ, VCO2 (mL/RR), VE/VO2, FET O2, and Ox. Util. Fact. values at the third minute after the test. No significant dynamics was found in RR, VE/VO2, and FET CO2 through all stages of the experiment.

Our results obtained indicate complicated diversified patterns in the gas analysis and the external respiration readjustments in response to hypoxic- & hypercapnic exposure. We examined the peak values and found 7.4±0.2 % of carbon dioxide and 12.2±0.2 % of oxygen in the collecting bag after test, which made it only possible to use their own metabolic CO2 to make hypercapnic effects. Under the rebreathing test, the subjective breathing patterns tended to change with running VE ВТPS (pulmonary ventilation) due to higher Vt ВТPS and with no changes in RR compared to the baseline values.

The growth of Vt ВТPS occurs by using reserve volume of inhalation and exhalation, i.e., by a stronger contraction of the respiratory muscles, which provides deeper breathing. Greater importance of Vt ВТPS in pulmonary ventilation (VE ВТPS) indicates more reserve capabilities of the respiratory system and some effective patterns of adaptation which develop under loads [1]. The rebreathing peak significantly (by 70%) raised the CO2 values, which remained high in the recovery period in comparison with the resting values. Carbon dioxide being the final product of metabolism develops biochemical reactions since it is a pronounced stimulator of the central nervous system, hemodynamics, the vascular tone, and it is also important in the regulation of the gas exchange and respiration functions [13, 14, 15].

The gas exchange chemo-regulation mechanism controls respiratory system functioning and is aimed at maintaining the proper partial pressure of carbon dioxide in the red blood cell mass [16]. It is known that the fastest way to reduce the amount of CO2 in the body is to increase the ventilation function of the lungs [17]: chemoreceptors produce a significant effect on the broncho-pulmonary system activity, since they sensitively react even to slight changes in the chemical composition of blood washing them [18]. Being in their excited state, the chemoreceptors contribute to meeting the body metabolic needs by intensifying the respiratory system activity [18]. The VE/VCO2 indicator is a carbon dioxide ventilation equivalent; it reflects the ventilation needs for a specific amount of produced CO2 [19] and is currently considered as a marker of the chemo-reflecting sensitivity [20]. Changes which occur in a linear relation between VE and carbon dioxide removal (VE/VCO2) are currently used for estimating the ventilation efficiency [21].

In our survey, we observed the test-peak-accelerated (by 66 %) pulmonary ventilation that fully removed excessive carbon dioxide, which was confirmed by zero dynamics in the carbon dioxide ventilation equivalent in response to the rebreathing test. The increased ventilation per minute (VE) and the CO2 removal during the hypoxia-hypercapnia test are necessary for the homeostatic pH control in the body. Besides, that eventually reduces arterial PCO2 [17]. The oxygen consumption rate (VO2) is the main characteristic of the gas exchange. However high VO2 values reflect metabolic processes in the body rather than the external respiration and thus indicate a significant intensification of metabolism [22]. A hypoxic-hypercapnic exposure increases the body oxygen needs, which can be seen in an increased (by 34 %) rate of its consumption and the lowered VO2 values in the recovery period as compared to the baseline.

G. Karaterzi in some works (2011) also indicates a reduction in the oxygen consumption after hypoxic training, which suggests a more efficient use of oxygen by tissues, a lower metabolic demand [23], and evaluates tissue adaptation to lack of O2 [24]. According to F. Joulia (2002), hypoxemia and hypercapnia are associated with a reduced oxygen uptake [25].

The oxygen ventilation equivalent (VE/VO2) values determine respiratory needs at a given level of the O2 utilization [19]. In our survey, we obtained fairly low resting oxygen equivalent values (VE/VO2), significantly lower than those given in other authors’ studies [26]. At the same time, we observed higher VE/VO2 in response to the rebreathing test, which indicated the lowered respiration efficiency and was confirmed by the decreasingly low oxygen utilization factor. The VE/VO2 and oxygen util. factor dynamics is associat-
ed with the gas exchange energy metabolic indicators that is significantly reduced in the recovery period (vs. the baseline and the peak values): Kcal, REE, and REE/Pred which are commonly used in scientific papers abroad as some criteria for metabolic adaptation, as well as the so called price of energy costs in people living in different climatic and geographical regions including the circumpolar zones [27]. In this context, our data are fully consistent with those indicated by those authors who associate low REE values after hypoxia with the energy conservation needs in order to prevent further energy loss due to a reduced oxygen consumption, which can be caused by lowered excitability in hippocampal neurons in the brain after hypoxia [28].

The overall dynamics of the carbon dioxide removal rate does not correspond to the changes in the oxygen consumption rate described above. At the peak of the test, VCO₂ increases by 70% versus the 34% increase in the rate of the oxygen consumption. The VCO₂/VO₂ ratio reflected in the respiratory quotient (RQ), which assesses the metabolic processes, tends to rise at the peak, but not to reduce in the recuperation.

Discussion and conclusions

The gas exchange readjustments caused by the combined hypercapnia and hypoxia exposure influence breathing patterns, which can be seen in accelerated metabolism at the peak states of the test and the reduction in the recovery. The respiration system demonstrates more pronounced pulmonary ventilation with deeper breathing as compared to the baseline value at any stage of the recovery period, up to the 3rd minute. In addition, our study has confirmed the previously described findings about the reduced oxygen consumption after the hypoxia and hypercapnia test. The ventilation equivalent values demonstrate their multidirectional changes, with no significant dynamics for carbon dioxide at all stages of the study, but significant and pronounced changes for oxygen observed in the post-rebreathing and recovery periods.

Statement on ethical issues

Research involving people and/or animals is in full compliance with current national and international ethical standards.

Conflict of interest

None declared.

Author contributions

The authors read the ICMJE criteria for authorship and approved the final manuscript.

References

Cognitive, emotional-affective, anxiety and autonomic disorders in patients with a new coronavirus infection (covid-19) in the acute period

Konstantin N. Melnikov*, Viktor P. Kondratyev
Medical University “Reaviz”, 443011, Russia, Samara, Chapaevskaya st, 227
*Corresponding author: vstenv@gmail.com

Abstract
The purpose of this work is to identify neuropsychiatric functions in patients at Department No. 1 responsible for medical care of patients with a new coronavirus infection at the Samara City Hospital No. 7. Appropriate scales and questionnaires were used for this purpose. Cognitive impairments were found in 86% of the cases, emotional-affective impairments of varying severity in half of the subjects, an increase in reactive and personal anxiety was revealed, and vegetative disorders were observed in 78% of the subjects. Based on these data, it can be assumed that the new coronavirus infection affects the functioning of the nervous system of patients.

Keywords
New coronavirus infection, COVID-19, Cognitive skills, Emotional-affective functions, Anxiety, Autonomic nervous system

Introduction
The outbreak of the COVID-19 pandemic caused by SARS-CoV-2 has shown to the medical community that the possible clinical manifestations of diseases caused by viruses of the Coronaviridae family are not well understood. The damage to the nervous system and its higher functions has been particularly poorly studied, while the incidence of such damage is quite high.

According to recent studies, COVID-19 causes damage to the central nervous system, including headache, confusion, and disorientation in 25% of the cases [1]. According to another study, neurological disorders in the new coronavirus infection are observed in 36.5% of the cases, including disor- dering of taste and olfactory functions, myalgia, headaches and acute disorders of cerebral circulation, dizziness, mental dysfunctions, encephalitis, ataxia, the Guillain–Barré syndrome and Miller Fisher syndrome [2].

The study of magnetic resonance imaging (MRI) data in patients with anosmia (loss of smell) and ageusia (loss of gustatory sensitivity) did not reveal changes in the olfactory bulbs on both sides [16]. According to the results of 18 autopsies of COVID-19, those patients who died in April 2020, based on autopsy data, showed cerebral ischemia. During the same study, no evidence of direct viral infection was found, but only minimal inflammatory changes were demonstrated. Thus, it can be assumed that SARS-CoV-2 does not penetrate the blood-brain barrier [3]. This is also confirmed by the study of cerebrospinal fluid (CSF), which has been carried out in patients with neurological disorders against the background of the new coronavirus infection using the method of polymerase chain reaction with reverse transcriptase; as a result of this study, SARS-CoV-2 has not been detected in the CSF [15].

To date, the mechanisms of the pathogenesis of damage to the nervous system are not fully understood. It is assumed that the mechanisms of involvement of the central nervous system in the pathological process in COVID-19 are different, and the authors hereof identify 3 most likely options: 1) The cytokine storm increases the BBB permeability, which promotes the penetration of viruses, bacteria, immune cells, toxic metabolites and pro-inflammatory agents into the structures of the central nervous system. 2) It is known that SARS-CoV-2, like SARS-CoV-1, can interact with the type 2 ACE receptors, which can lead to direct damage to neurons without development of pronounced inflammation; 3) The direct effect of the virus on the respiratory center in the brain stem can cause shortness of breath, characteristic of COVID-19, without severe lung damage [4]. Neurological disorders in the new coronavirus infection can
be both the first manifestations of the disease and its complications that occur 24 hours after the onset of the disease or later [12].

Psychiatric disorders associated with the new coronavirus infection are even less covered by the scientific literature. Most of the articles are devoted to the level of anxiety disorders in the population against the background of the pandemic and self-isolation, acute stress disorders and as the vegetative syndrome after the infection. A systematic review and meta-analysis of the articles on the relationship between mental disorders and the coronavirus infection, including MERS-CoV, SARS-CoV-1 and SARS-CoV-2, suggests that confusion and psychomotor agitation are common among mental disorders for the acute period of the coronavirus infections. There is growing evidence of how the forced self-isolation has a negative impact on mental health. 53.8% of people in isolation stated that their psychological state had greatly deteriorated [19].

It was decided not to consider in this work acute cerebrovascular accidents against the background of the new coronavirus infection in connection with the study format, which is not suitable for this purpose, as well as a sufficient number of works devoted to this topic.

Therefore, we considered it important to pay attention to the study of the effect of SARS-CoV-2 on the nervous system, more specifically on the cognitive, emotional-affective, anxiety and autonomic functions of the nervous system.

Materials and methods

For this purpose, a survey was conducted to cover 50 patients diagnosed with “the new coronavirus infection” in the acute period at the age of 22 to 59 years. The median age was 48.6 years. According to the WHO classification (2016), we analyzed our evidence data as follows: young age (18–44 years) in 28% of the subjects (n = 14), average age (45–59 years) in 72% of the test subjects (n = 36). There were 38% of men (n = 19) and 62% of women (n = 31) among them. The degree of lung damage according to the CT imaging was determined as CT-1 (up to 25% of the lungs were affected) in 63% of the patients, CT-2 (26–50% of the lungs were affected) in 27% of the patients, CT-3 (51–75% of the lungs were affected) in 10% of the patients, CT-4 (more than 75% affected) in 0% of the recorded cases. According to the results of the PCR examination of the oropharyngeal smear, SARS-CoV-2 DNA was detected in 50% of the cases (n = 25). No SARS-CoV-2 DNA was detected in 50% of the recorded cases (n = 25). At the same time, the condition of all patients corresponded to the moderate course of COVID-19 in accordance with the provisional guidelines for the prevention, diagnosis and treatment of the new coronavirus infection (COVID-19), version 10. The study was conducted during March–April 2021.

As mentioned above, it is assumed that the causes of disorders of the nervous system in the new coronavirus infection are different and include the following: the direct effect of the virus on the nervous system, the systemic inflammatory response syndrome, the multiple organ dysfunction, the cerebral vascular thrombosis, the endothelial dysfunction, etc. [13, 14]. In addition, hypoxemia against the background of respiratory failure, exacerbation of concomitant chronic diseases, and cognitive impairments of old age could lead to impaired functioning of the nervous activity. The most frequent comorbidities in our study were hypertension and type 2 diabetes mellitus. Also, the patient’s unwillingness or deliberate distortion of the answers when completing the questionnaires might lead to unreliable results. In order to exclude these factors as much as possible, some eligible criteria for inclusion and those from the study were developed.

We applied the study inclusion criteria as follows:
- Men and women aged 18-59 years, hospitalized from one to three days ago, who did not receive specific treatment with antiviral drugs within the above time span;
- Patients who signed their written informed consent to participate in the study.

At the same time, we used the criteria for an exclusion from the study as given below:
- Age under 18 and over 59 years old;
- Pregnancy;
- Severe concomitant somatic and / or psychiatric pathology;
- Severe and extremely severe course of Covid-19;
- Refusal to sign written informed consent.

The following methods were used for the survey: MoCA, Montreal Cognitive Assessment - the Montreal scale of cognitive assessment (Maximum score of 30; a score of 26 and over is considered normal, a score less than 26 indicates moderate cognitive impairment); the McNair and Kahn Self-Assessment Memory Inventory (Cognitive impairment should be assumed with score> 42); Beck Depression Scale II (score 0–13 is considered a variation of the norm, 14–19 indicates mild depres-
Research results

In our study we have obtained some interesting results. Thus, 86% of the patients have moderate cognitive impairments according to the MoCA data (mean score 22.1, PCR “+” 22.56, PCR “−” 21.64), and only 13% of the individuals noted cognitive impairment in them according to the questionnaire of self-assessment of memory by McNair and Kahn (average score 26.12, for PCR “+” 28.26, for PCR “−” 24.08). The Beck Depression Scale (mean score 9.06, PCR “+” 7.87, PCR “−” 10.32) showed a norm in 53% of the cases, mild depression in 27% of the recorded cases, moderate depression in 13% of the studied cases and severe depression in 7% in the cases. The Spilsberger-Hanin Anxiety Scale examines both reactive and personal anxiety, and we have obtained the following data on the levels of reactive anxiety in the patients with COVID-19 (average score 46.61, PCR “+” 46.58, PCR “−” 46.65): low in 4% of the patients, moderate in 44% of the examined cases, and high in 52% of the patients. The levels of personal anxiety in patients with COVID-19 (mean score 42.97, PCR “+” 42.33, PCR “−” 43.68) are reported as follows: low in 6% of the patients, moderate in 54% of the patients, and high in 39% of the cases. The syndrome of autonomic dysfunction according to the questionnaire by A.M. Wein is observed in 78% of the patients, the norm in 22% of the individuals (mean score 24.24, for PCR “+” 23.43, for PCR “−” 25.09). Average scores and percentage of the results obtained are presented in Table 1 and Table 2 given herein.

The high incidence of mild cognitive impairment requires further careful consideration of the structure of these cognitive impairments. The Montreal Cognitive Assessment Scale (MoCA) consists of several tasks, which are divided according to the areas of cognitive functions indicated below: visual-constructive skills (the maximum score of 5), naming (the maximum score of 3), memory (no scores), attention (the maximum score of 6), speech (the maximum score of 3), abstraction (the maximum score of 2), delayed reproduction (the maximum score of 5), and orientation (the maximum score of 6).

As a result of our survey, the following results were obtained: visual-constructive skills (average score – 3.88), naming (average score – 3), memory (no scores), attention (average score – 5.24), speech (average score – 1.86), abstraction (average score – 0.96), delayed reproduction (average score - 1.46), and orientation (average score – 5.7). It can be seen that the functions such as naming and orientation remain practically unaffected. The areas of cognitive function most affected are abstract thinking and delayed reproduction. Since these functions are carried out by the frontotemporal areas of the cerebral cortex, it can be assumed that these areas are involved in the pathological process in COVID-19.

The discussion of the results

To understand and evaluate the results obtained, it is necessary to compare them with the indicators in the population. For this purpose, data from large population studies can be consulted. Thus, the average score on the MoCA scale range among French-speaking people in Quebec was reported to be 26.4 [5]. In the Moroccan population, the average score on the MoCA scale range among people from 18 to 39 years old was 28.06, and among people from 40 to 59 years old it was reported to reach 27.86. [6]. At the same time, it should be noted that the average score according to the MoCA scale among patients with the new coronavirus infection in the acute period was recorded to be 22.1. Thus, a decrease in cognitive functions can be seen in patients hospitalized with diagnosis of “the new coronavirus infection” in the acute period, in comparison with the above population studies. Even in asymptomatic patients with COVID-19, cognitive decline has been observed. [7]

The data obtained using the McNair and Kahn Self-Assessment Memory Questionnaire show the opposite to the MoCA scale. It can be assumed that the reason for this is the duration of cognitive impairment. The questions presented in the questionnaire, such as “I forget phone numbers,” “I forget the names of my old acquaintances,” and others, require a long-term presence of cognitive impairments in order to notice by the subjects their cognitive deficit and be able to more confidently answer these questions.
The average BDI scores in the studied populations in Europe ranged from 4.18 (Norwegian men aged 18–40 years) to 10.69 (Liverpool women aged 41-65 years) [8]. The mean BDI score in a study in Denmark was reported to be 10.6 [9]. The high level of depression in men aged 25–64 years of the Tyumen region was 4.6%, the average level of depression in the same group was recorded to be 19% [21]. Among the residents of the Samara region aged 19 to 68 years, in relation to risk factors for chronic non-infectious diseases, the depression syndrome was observed in 25.2% of the surveyed individuals, including the subclinical level of depression in 21% the cases, and the clinical level in 4.2% of the recorded cases [20].

“Moderate to severe” anxiety in a study by Susan Bri- man among 6189 people aged 25 to 65 was experienced...
by 12.5% of the men and 19% of the women [10], while the moderate and high level of anxiety among patients with the new coronavirus infection in the acute period was observed in more than 80% of the subjects. This is not surprising as these patients have many reasons for concern and fear: facing an inexperienced disease, the danger of which everyone around is talking about, including television, the Internet, word of mouth; hospitalization in the department, where all medical personnel are dressed in anti-plague suits. All this initiates fear and anxiety in patients concerned about their lives, their future and their ability to work. This is also evidenced by data from a survey conducted among the population in the late 2020 – early 2021. An increased level of anxiety was noted therein, moreover, in the age group from 18 to 24 years old, the high level of personal and situational anxiety was recorded to be 76.7% and 72.6%, respectively [17], while in 2014 the frequency of an increased level of anxiety among the population was 46.3% on average [18]. Thus, it is impossible to draw an unambiguous conclusion about what caused anxiety among patients with the new coronavirus infection in the acute period: the influence of the SARS-CoV-2 virus or some external stressors in the form of the pandemic, self-isolation and general alertness. However an increase in the level of anxiety among this group of people is indeed observed.

The postural orthostatic tachycardia syndrome and other autonomic disorders can accompany the new coronavirus infection in previously healthy patients and 6-8 months after the acute period [22]. The study of autonomic disorders under the post-Covid syndrome demonstrated the predominance of the sympathetic activity of the autonomic tone, autonomic response and autonomic support of the activity [23]. But the autonomic disorders in patients with the new coronavirus infection are present not only in the recovery period, but also in the acute period. So, using the scale for assessing autonomic changes, developed by Professor Alexander Moiseevich Vein, the vegetative dystonia syndrome is detected in 78% of the subjects. In the population, the vegetative dystonia syndrome is observed in 56% of the cases. Also, the severity of anxiety disorders increases in proportion to the severity of autonomic disorders [11].

Conclusion

Conclusions from our study can be drawn as follows:

- 86% of the patients with new coronavirus infection COVID-19 have moderate cognitive impairment, especially in case of “abstraction” and “delayed thinking” on the MoCA scale;
- Subjective feelings of memory problems are present in 13% of the COVID-19 patients;
- Emotional-affective disorders of varying severity are observed in half of the subjects;
- An increased level of reactive anxiety is found in comparison with the personal anxiety, as well as an increased level of anxiety in general;
- Autonomic dysfunction is observed in 78% of the patients with the new coronavirus infection.

Unfortunately, it should be noted that our work has some limitations. So, it was not possible to investigate the above functions in the examined patients before their disease. There is no way to unequivocally identify the effect produced by SARS-CoV-2 on the nervous system. It is all the more difficult to talk about the pathogenesis of this influence. There is still a lot of work to be done in the future, including checking whether the impairments of the cognitive, emotional-affective, anxiety and vegetative spheres persist after the acute period of the new coronavirus infection, or whether these impairments are transient. Also, it is necessary to investigate the relationship between the post-Covid syndrome and the disorders in the acute period. The world’s population, and especially the health care community, will still face the consequences of COVID-19, and our task is to contribute in order to be prepared to meet the challenge.

Statement on ethical issues

Research involving people and/or animals is in full compliance with current national and international ethical standards.

Conflict of interest

None declared.

Author contributions

The authors read the ICMJE criteria for authorship and approved the final manuscript.

References


7. Cognitive assessment in asymptomatic COVID-19 subjects Sridhar Amalakanti; Kesava Venkata Raman Arepalli; Jyothi Priya Jillella. Received: 4 September 2020 / Accepted: 26 January 2021 Indian Virological Society 2021


10. Normative data for the Hospital Anxiety and Depression Scale. Suzanne Breeman, etc.

11. Yakhina F.F. Population studies of the syndrome vegetative dystonia. 2005


Photometric recorder of the carotid artery pulse wave with a system for stabilizing the clamping force

Alexey V. Berdnikov*, Georgy V. Matrosov

Kazan National Research Technical University named after A. N. Tupolev, 420111, Russia, Kazan, Karl Marx st., 10.

*Corresponding author: alex-berd@mail.ru

Abstract
Preventive monitoring, which increases the possibility of diagnosing and curing a disease at an early stage, is interesting and relevant in medical diagnostics. Recently, both minimally invasive and non-invasive diagnostic techniques have become widespread. They include the areas of cardiographic, encephalographic and myographic analyzers designed to study the state of the heart, the brain and striated muscles. The study of the functioning of the smooth muscles of the gastrointestinal tract is carried out using gastro-enterography methods. To obtain an integral assessment of the effectiveness of the circulatory system, the plethysmography method can be successfully applied, which allows modeling vascular tone and blood flow using models of hydrodynamics and the theory of elasticity. The present article analyzes the range of static (or dynamic) informative hemoparameters that allow diagnosing at the initial stage arterial hypertension, aortic insufficiency or stenosis, atherosclerosis, diabetes mellitus and some other diseases. The photometric reflex absorption in the infrared range, followed by an assessment of blood flow in the aortas and arteries with recording of the shape of pulse waves were used as methods in our research work. The result of the work is a method and a system of computer control of the rod current acting on biological tissues (using the Bluetooth protocol, LabView environment, virtual digital filters and biomedical data archives) that allows non-invasively recording of the parameters of the carotid artery pulse wave.

Keywords
System, Stabilization, Pulse wave, Carotid artery

Imprint

Introduction
Non-invasive and technologically realizable methods for diagnosing the cardiovascular system are of great interest to the scientific medical community due to the availability of this type of research to the public in general (Omelyanovskiy, Avksentieva, et-al 2021) that increases the number of patients receiving treatment at the initial stage; including that with the help of telemedicine (Naberezhnaya, Zakharov, 2021; Totskaya, Pokrovskaya, 2018).

The plethysmography method belonging to this group of studies in its practical application is based on the concept of the vascular tone and the blood flow as the physiological functions, to the study of which elements of the theories of elasticity and hydrodynamics are applicable. This method is capable to produce a full range of informative hemostatic and hemodynamic parameters, which makes it possible to use it in the initial diagnosis of diseases such as arterial hypertension, aortic insufficiency, atherosclerosis, aortic stenosis, diabetes mellitus, Bottalo’s duct non-closure, obliterating endarteritis and others (Usanov, Skripal, et-al 2009).

Theoretical foundations and reference literature analysis
An introduction of modern methods and technologies for assessing the quality of medical care (Zhelznyakova, Seryapina, et-al 2020; Nicolay, Purkayastha, 2012; Harvey, 1996) also requires some new specific diagnostic techniques.

When using single signs for diagnostic studies on the pulse waveform, the most informative are the following: the absence of a dicrotic notch and the difference in the volumetric pulse on the arms and legs (Okorokov, 2007). Recording of pulse fluctuations can be carried out using photometric reflex-absorption sensors (Whitenett, Stewart, et-al 2003; Posani, Tripathi, et-al 2006) operating in the infrared range, the signal of which is proportional to the blood filling of the arterial vessels, lying under the sensor, at every moment of time.
Variants of devices for recording a pulse wave which exist today are designed as a clamping device attached to a finger or a toe, and they operate with transmitted radiation. This approach to the design of a plethysmographic meter, operating on reflected radiation, is associated with a significant drawback, which consists in the inability to control the clamping force of biological tissue containing blood vessels; this involves some changes in the blood flow in the investigated area and, as a consequence, leads to an increase in the measurement error. In addition, to assess the performance of the heart and the root sections of the circulatory system (for example, (Actual Problems of Prevention, 2015), it is of particular interest to assess blood flow in large aortas and arteries, as in vessels adjacent to the heart. This assessment requires recording pulse waves on large arteries, which cannot be ensured with the use of a clothespin-like sensor.

Materials and methods

Based on the above, the vector of scientific and engineering synthesis becomes clear: it is aimed at obtaining an alternative, improved, method for collecting and analyzing the parameters of pulse waves (Simonenko, Tuchin, 2007; Shurygin, 2000). An intermediate stage of this synthesis is the concept of an analyzer of the parameters of the carotid pulse waveform with a system for stabilizing the clamping force (Novikov, Matrosov, Berdnikov, 2018).

The design of the device includes two main parts, namely, a housing and a measuring head. Sensitive elements are located inside the head: a strain-resistive transducer and a photometric sensor. In this case, the measuring head can move in the housing along its axis. This design solution provides for the kinematic system necessary for the implementation of the mechanism for stabilizing the force of pressing the head against the tissues.

The mechanism for stabilizing the force of pressing the head to the tissues allows avoiding changes in the blood flow in the examined area of biological tissue and assumes that the rod of the electromagnet is rigidly connected to the measuring head, and the body of the electromagnet is rigidly connected to the body of the device. This mechanism is realized with the use of a solenoid electromagnet, a control logic circuit, a current setting system and a strain gauge circuit (Dachik, Prigorovsky, Khurshudov, 1989). Thus, by controlling the strength of the current in the winding of the electromagnet, it is possible to control the force with which the rod will act on the biological tissue.

Results and discussion

The following sequence of research steps is assumed:

1. Smooth application of the sensitive surface of the device onto the area of the carotid artery location.
2. Gradual increase in the pressure applied to the handle in the longitudinal direction until a signal appears that the device is capable of delivering readings, that is, it enters its proper operating mode. In this case, the head rod will be in its central position and, therefore, has an equal stroke reserve for stabilization, both for clamping and removing the device.
3. Holding the device in its operating position, i.e. pressing onto the handle (if possible) with a force ap-
proximately equal to that which is applied at the moment the signal appears that the device enters its operating position.

4. Check-up and analysis of data delivered by the device.

The device, in addition to displaying brief hemodynamic data on the built-in display, allows streaming data to a PC via Bluetooth. The data obtained by the PC is then interpreted as a pulse wave graph using the capabilities provided by the NI LabView software package (Travis, Kring, 2008). In this case, it is possible to capture “raw data”, the filtering of which is carried out directly in the LabView environment by means of virtual digital filters (see Figure 3 herein). The pulse wave graph can be saved by a workstation or by a server in a medical office in order to archive biomedical data (Novikov, Berdnikov, Burmistrov, 2016).

A browser solution is also possible that significantly expands the scope of the device (Berdnikov, Ynusov, et-al, 2019), extending the accessibility, as well as computer simulation (Kaziev, 1990) in a graphic mode.

Conclusion

Thus, this offered device allows non-invasive recording followed by an analysis of the parameters of the carotid artery pulse waveform, while the accuracy of the analysis is increased due to the use of the specific system for stabilizing the force of pressing the measuring head to the biological tissue.

The proposed design solution makes it possible to implement the required kinematic system for stabilizing the force of pressing the head against the biological tissues.

Statement on ethical issues

Research involving people and/or animals is in full compliance with current national and international ethical standards.

Conflict of interest

None declared.

Author contributions

The authors read the ICMJE criteria for authorship and approved the final manuscript.

References

2. Embankment IB, Zakharov DA. The role of the regional telemedicine center in the provision of medical care. Medical technologies. Evaluation and Se-
3. Totskaya EG, Pokrovskaya OB. Telemedicine as a mechanism for ensuring the availability of high-tech medical services and innovative technologies in health care. Emergency Doctor. 2018;6:71-7. [in Russian]


Impact of 3D transesophageal echocardiography assessment of mitral valve on short-term outcome of Balloon Mitral Valvuloplasty using additional quantitative parameters

Ibrahim Ahmed Elsawah1*, Ali Mohamed Al Amin2, Abdel-Mohsen Moustafa Abdo3, Mohamed Osama Taha1, Essam Ahmed Khalil4

1Department of Cardiology, National Heart Institute, Cairo, Egypt.
2Cardiology Department, Faculty of Medicine, Al Azhar University, Cairo, Egypt.

*Corresponding author:
dr.sawah@yahoo.com
+00201026600390

Abstract

Aims: The goals of this prospective observational study were to identify quantitative morphological factors that might aid in the prediction of PBMV outcome, as well as to define the function of 3D TEE in mitral valve evaluation.

Material and Methods: The trial comprised seventy individuals who had isolated severe rheumatic Mitral Stenosis (MS) who were scheduled for PBMV between October 2017 and March 2021. Trans-thoracic echocardiography (TTE) was used for pre-procedural evaluation. Posterior to Anterior Leaflet Length Ratio (P/ALLR), Commissural Area Ratio (CAR), Doming Height (DH), and Calcification Score (Ca score) were also assessed using 2D and 3D Trans-esophageal Echocardiography (TEE). Following that, all patients were monitored for three months.

Results: We have had 50(71.5%) successful procedures and 20(28.5%) unsuccessful procedures. The additional parameters showed highly significant difference between successful and un-successful groups (P-value < 0.001). The cut-off points that predict successful procedures were; P/A LLR > 0.6 and > 0.55 by 2-D and 3-D TEE respectively, CAR ≤ 1.28 and ≤ 1.25 by 2-D and 3-D TEE respectively, DH > 1.2 cm by 2-D TEE, and Ca Score ≤ 2.

Conclusion: In our research, we discovered that these characteristics may predict the result of PBMV and contribute value to the semi-quantitative methodologies we adopted to use.

Keywords
3D Transesophageal echocardiography, Balloon Mitral Valvuloplasty, Additional quantitative parameters

Imprint

Introduction

Rheumatic heart disease (RHD) is one of the most frequent cardiac disorders in poor nations, where it is the second leading cause of morbidity and mortality after atherosclerotic vascular diseases [1]. The most frequent valve lesion encountered in chronic RHD is MS, which often presents as exertional dyspnea and symptoms of right heart failure caused by pulmonary hypertension [2]. For most people with severe MS, PBMV is regarded a better option than surgery [3]. The pre-procedural echocardiographic score, which reveals the degree of mitral valve morphologic defects, is the strongest predictor of immediate outcomes [4]. For the examination of mitral valve morphology prior to PBMV, a two-dimensional (2D) echocardiographic total scoring approach (Wilkins’ score) has been presented. Total scores for leaflet motion, leaflet thickness, valvular calcification, and subvalvular disease are used in this procedure [5]. Although this scoring approach has been frequently utilized because of its simplicity, each component grading remains semi-quantitative, sensitive to observer variability, and less accurate in identifying patients with scores in the mid-range [6]. Several criteria, such as asymmetrical commissural affection and absolute leaflet displacement in diastole, have been postulated for predicting Mitral Regurgitation (MR), although the ideal combination of characteristics predicting outcome variables has yet to be determined [7]. TEE is a highly effective diagnostic tool for assessing heart structure and function [8]. Unlike 2D TEE, which employs typical imaging planes, 3D TEE uses volume datasets to provide a more comprehensive assessment of mitral valve (MV) anatomy and morphology from numerous angles [9].

This study implies that these morphologic quantitative characteristics will be a straightforward and independent tool for predicting PBMV outcome.
Aim of the work

The goal of this study is to identify quantitative morphological parameters that may be used to predict success following PBMV, as well as to define the function of 3D TEE in mitral valve evaluation.

Material and method

The study included seventy patients, both sexes, over the age of 18, who had isolated severe MS with a Wilkins’ score ≤ 12, as well as non-existing or mild mitral MR, and who underwent PBMV between October 2017 and March 2021. All participants in the research provided informed consent. Any patient who had a contraindication for the procedure was ruled out. Each patient had a complete medical history taken including age, gender, diabetes, hypertension, NYHA class, clinical examination, and a 12-lead resting electrocardiogram (ECG). TTE examined the traditional indices for assessing MS severity, such as mitral valve area (MVA) by planimetry, peak and mean transmitial valve pressure gradients, MR severity with vena contracta (MRVC), and PASP, before and after PBMV. TEE was performed before the procedure to confirm grade of MR, rule out thrombus, and examine the mitral valve using the quantitative parameters listed below; in the mid esophageal long axis view, P/ALLR and DH were assessed as indicated in (Figure 1). Then, with the image fixed at end diastole after using the X-plane and 3D zoom modes, a transverse line was drawn connecting both annular sides and crossing the commissures, another line was drawn to define the mid-point of the first line, the areas on both sides of this point were traced, and the large area was divided by the small one to get CAR (Figure 2). P/ALLR was calculated by measuring the length of the anterior and posterior leaflets in the same image. Calcification Score (0-10) was assessed using Anwar Score; 1 point for calcification in one of the middle scallops vs 2 points if it appears in one of the commissural scallops [10]. After PBMV, success was defined as successful dilatation of the MV with an area (MVA) more than 1.5 cm², as well as no deterioration of the MR by more than one grade; MRVC ≤ 0.4cm. For three months, all patients were monitored for the necessity for MVR, re-do, NYHA, rhythm, and death.

Statistical Analysis

Data were gathered, edited, coded, and put into the IBM SPSS version 23 Statistical Package for Social Science. When the data was determined to be non-parametric, the quantitative data was presented as mean, standard deviations, and ranges rather than mean, standard deviations, and ranges. Qualitative factors were also given numerically and as percentages. The following p-values were judged significant: P-value more than 0.05 indicates non-significant (NS), P-value less than 0.05 indicates significant (S), and P-value less than 0.01 indicates highly significant (HS) (HS).

Results

According to the results of PBMV, patients were classified based on procedure success and the two el-
elements of procedure results separately; degree of dilatation and progression of MR.

The study included 70 patients; 57 (81.4%) were female and 13 (18.6%) were male. The mean age was 34.69 ± 8.65 years. There was only 9 (12.9%) diabetic patients and 10 (14.3%) were hypertensive. Regarding rhythm; 19 (27.1%) patients were AF (Table 1).

We have had 50(71.5%) successful procedures and 20(28.5%) unsuccessful procedures; 10 patients purely non-dilated, 9 patients were dilated but had worsening of MR and 1 patient was non-dilated and had worsening of MR (Table 2).

Only ECG rhythm had the significant difference between the two groups; 9 (45%) of unsuccessful patients had atrial fibrillation (AF) and 40 (80%) of successful patients were sinus (P-value 0.034). (Table 3)

Table 4 reveals that the mean value of Wilkin’s score showed a highly significant difference (P-values 0.001). The mean values of left atrial diameter (LAD) and peak pressure gradient demonstrated a significant difference between the two groups (P-values 0.027 and 0.045 respectively). However, there was no significant difference in MVA, mean pressure gradient, PASP, or MRVC. When the two components of success were evaluated independently, we discovered that baseline MVA differed significantly between the dilated and non-dilated groups (P-value 0.014), while it showed non-significant difference when compared with mitral regurgitation results (P-value 0.821).

The mean value of P/A LLR, CAR, and DH showed highly significant difference between successful and unsuccessful groups (P-value 0.000) (Table 5).

Table 1

<table>
<thead>
<tr>
<th>Age</th>
<th>Mean ± SD</th>
<th>34.69 ± 8.65</th>
</tr>
</thead>
<tbody>
<tr>
<td>Range</td>
<td>18 – 58</td>
<td></td>
</tr>
</tbody>
</table>

Table 2

<table>
<thead>
<tr>
<th>Procedure Success</th>
<th>Un Successful</th>
<th>Successful</th>
<th>No. = 20</th>
<th>No. = 50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dilatation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>11 (55.0%)</td>
<td>0 (0.0%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>9 (45.0%)</td>
<td>50 (100.0%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mitral regurgitation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>10 (50.0%)</td>
<td>50 (100.0%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>10 (50.0%)</td>
<td>0 (0.0%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3

<table>
<thead>
<tr>
<th>Basic data</th>
<th>Success</th>
<th>Test value</th>
<th>P-value</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Un Successful</td>
<td>Successful</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>Mean ± SD</td>
<td>36.25 ± 5.88</td>
<td>34.06 ± 9.52</td>
<td>0.956*</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>26 – 47</td>
<td>18 – 58</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td>Female</td>
<td>17 (85.0%)</td>
<td>40 (80.0%)</td>
<td>0.236*</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>3 (15.0%)</td>
<td>10 (20.0%)</td>
<td></td>
</tr>
<tr>
<td>DM</td>
<td>No</td>
<td>18 (90.0%)</td>
<td>43 (86.0%)</td>
<td>0.204*</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>2 (10.0%)</td>
<td>7 (14.0%)</td>
<td></td>
</tr>
<tr>
<td>HTN</td>
<td>No</td>
<td>17 (85.0%)</td>
<td>43 (86.0%)</td>
<td>0.012*</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>3 (15.0%)</td>
<td>7 (14.0%)</td>
<td></td>
</tr>
<tr>
<td>ECG rhythm</td>
<td>A</td>
<td>9 (45.0%)</td>
<td>10 (20.0%)</td>
<td>4.515*</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>11 (55.0%)</td>
<td>40 (80.0%)</td>
<td></td>
</tr>
<tr>
<td>C/O NYHA</td>
<td>NYHA I</td>
<td>1 (5.0%)</td>
<td>0 (0.0%)</td>
<td>2.819*</td>
</tr>
<tr>
<td></td>
<td>NYHA II</td>
<td>7 (35.0%)</td>
<td>22 (44.0%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NYHA III</td>
<td>12 (60.0%)</td>
<td>28 (56.0%)</td>
<td></td>
</tr>
</tbody>
</table>

*:Chi-square test; *: Independent t-test
As regard 3-D TEE measurements, the mean value of P/A LLR, CAR, and the median for Ca Score showed highly significant difference between successful and unsuccessful groups (P-value 0.000) (Table 6).

There were no deaths reported. Symptoms follow-up demonstrated better NYHA class in successful versus un-successful patients with highly significant difference (P-value 0.000). Regarding re-intervention, no patient (0%) in the successful group requires it, but 12 patients (60%) in the failed group require MV Replacement (MVR) with a highly significant difference (P-value 0.000), and 2 patients (10%) require re-do PBMV with a significant difference (P-value 0.023) (Table 7). The analysis of MVR patients revealed that three were solely non-dilated, one was non-dilated with MR, and eight had MR.

The cut-off points of 2-D TEE parameters that predict success were P/A LLR > 0.6 with 74.0% sensitivity and 100.0% specificity, CAR ≤ 1.28 with 90.0% sensitivity and 85.0% specificity, DH > 1.2 cm with 84.0% sensitivity and 95.0% specificity (Figure 3), and that for 3-D TEE parameters were P/A LLR > 0.55 with 84.0% sensitivity and 90.0% specificity. CAR ≤ 1.25 with 80.0% sensitivity and 95.0% specificity. Ca Score ≤ 2 with sensitivity 80.0% and specificity 75.0% (Figure 4).
Table 6
Comparison between pre-interventional 3D-TEE data according to procedure success.

<table>
<thead>
<tr>
<th>3-D TEE</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. = 20</td>
<td>No. = 50</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P/A LLR</td>
<td>Mean ± SD</td>
<td>0.52 ± 0.05</td>
<td>0.66 ± 0.09</td>
<td>Test value</td>
<td>-6.614*</td>
<td>P-value</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>0.45 – 0.7</td>
<td>0.49 – 0.9</td>
<td>Sig.</td>
<td>HS</td>
<td></td>
</tr>
<tr>
<td>CAR</td>
<td>Mean ± SD</td>
<td>1.33 ± 0.05</td>
<td>1.15 ± 0.10</td>
<td>Test value</td>
<td>7.152*</td>
<td>P-value</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>1.2 – 1.43</td>
<td>1 – 1.35</td>
<td>Sig.</td>
<td>HS</td>
<td></td>
</tr>
<tr>
<td>Ca score</td>
<td>Median (IQR)</td>
<td>4.5 (2.5–5.5)</td>
<td>1 (1–2)</td>
<td>Test value</td>
<td>-4.779#</td>
<td>P-value</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>0 – 7</td>
<td>0 – 4</td>
<td>Sig.</td>
<td>HS</td>
<td></td>
</tr>
</tbody>
</table>

* Independent t-test; †: Mann Whitney test

Table 7
Comparison of three months follow-up data according to procedure success.

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Un Successful</th>
<th>Successful</th>
<th>Test value</th>
<th>P-value</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>NYHA</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>NYHA I</td>
<td>3</td>
<td>15.0%</td>
<td>44</td>
<td>88.0%</td>
<td>42.875</td>
</tr>
<tr>
<td>NYHA II</td>
<td>5</td>
<td>25.0%</td>
<td>6</td>
<td>12.0%</td>
<td>36.207</td>
</tr>
<tr>
<td>NYHA III</td>
<td>6</td>
<td>30.0%</td>
<td>0</td>
<td>0.0%</td>
<td>5.147</td>
</tr>
<tr>
<td>NYHA IV</td>
<td>6</td>
<td>30.0%</td>
<td>0</td>
<td>0.0%</td>
<td>3.615</td>
</tr>
<tr>
<td>Rhythm</td>
<td>A</td>
<td>9</td>
<td>45.0%</td>
<td>10</td>
<td>20.0%</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>11</td>
<td>55.0%</td>
<td>40</td>
<td>80.0%</td>
</tr>
<tr>
<td>Need MVR</td>
<td>No</td>
<td>8</td>
<td>40.0%</td>
<td>50</td>
<td>100.0%</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>12</td>
<td>60.0%</td>
<td>0</td>
<td>0.0%</td>
</tr>
<tr>
<td>Need Redo</td>
<td>No</td>
<td>18</td>
<td>90.0%</td>
<td>50</td>
<td>100.0%</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>2</td>
<td>10.0%</td>
<td>0</td>
<td>0.0%</td>
</tr>
<tr>
<td>Death</td>
<td>No</td>
<td>20</td>
<td>100.0%</td>
<td>50</td>
<td>100.0%</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>0</td>
<td>0.0%</td>
<td>0</td>
<td>0.0%</td>
</tr>
</tbody>
</table>

*: Chi-square test

Figure 3: The Receiver-Operating Characteristic (ROC) curve of 2-D TEE measurements
Discussion

According to the procedure success definition, MVA was measured by planimetry to be greater than 1.5 cm² with no more than a one-grade rise in MR. Our study found that 20 procedures (28.5 percent) were unsuccessful, which is quite similar to Rifaie et al., study, which found that 30 percent of patients were unsuccessful [11]. In addition, Nunes et al., discovered (35 percent) of patients with sub-optimal outcomes [12]. Individuals with AF had a considerably poorer result than patients with sinus rhythm, according to Fatkin et al., (P-value 0.002) [13]. While Nunes et al., found no significant difference in rhythm (P-value 0.424), they did find a highly significant difference in age; the suboptimal group was elder (P-value 0.001) [12]. This might be explained by the fact that the mean age in our study was 34.69±8.65 years, whereas in Nunes et al., study it was 57±16 years [12]. Shaw et al., reported that PBVM produces good results in individuals under the age of 55, but the improvement is less maintained in the elderly [14]. Mailer et al., found that lower baseline MVA, larger trans-mitral gradient, and LAD were predictors of unsatisfactory results and the development of MR following PBVM [15], which was consistent with our findings. Nunes et al., found a highly significant difference in Wilkins score, baseline MVA, CAR, and DH between the two groups (P-value 0.001) [12]. Reifart et al., dilated in vitro 15 excised MV from MS patients who received MVR and discovered a 20% rate of leaflet rupture. They proposed that MR following PBMV is determined by the symmetry of morphologic alterations rather than their severity [16]. According to Rifaie et al., calcification and sub-valvular illness are the only independent predictors of immediate post-procedural prognosis [11]. Anwar et al., discovered that leaflet mobility and sub-valvular thickness as 3-D score components were independent indicators of procedural success (P-value 0.004 and 0.04 respectively) [10]. All of the previous investigations agreed with our findings when the quantitative evaluation of leaflet mobility was stated as maximal leaflet displacement; DH, and when valve thickness and fibrosis was expressed as P/A LLR and CAR. According to Mahfouz, P/A LLR is significantly correlated with PBVM outcome, with greater ratios producing much better results [17]. In terms of calcification, we assessed it using 3-D TEE and assigned a score in the same manner as Anwar et al., who proposed a new score for assessing MV, reported that 3DE could predict the extent and distribution of calcification in each scallop from a single short axis cut plain, and discovered that calcification was
the only independent predictor of the development of significant MR [10]. Shakil et al., found a significant difference in calcium detection and evaluation between 2-D and 3-D echocardiography; 2DE detected calcification in 26 (52 percent) of cases versus 35 (70 percent) of cases with 3-DE (P-value 0.002) [18]. According to Sutaria et al., and Messika-Zeitoun et al., calcification of commissures is a powerful predictor of outcome following PBMV because it influences the degree of commissural splitting [19,20]. Our findings reveal that 90 percent of patients with deteriorating MR require MVR, but only 13.6 percent of non-dilated individuals require MVR, indicating that severe MR is a better predictor of patients’ symptoms and need for MVR than poor MVA. Nunes et al., discovered that the event-free survival rate at 2 years was only 13% in patients with moderate MR against 62 percent in those with mild MR (P0.001), and the rate of MVR was significantly greater in individuals with moderate MR (46 percent versus 9 percent, P0.001) [12]. In our investigation, the cut-off values for quantitative parameters that indicate success were as follows: P/A LLR >0.6 and >0.55 by 2-D and 3-D TEE, CAR ≤1.28 and ≤1.25 by 2-D and 3-D TEE, and DH > 1.2 cm by 2-D TEE. It was virtually identical to Nunes et al., who concluded that DH > 12mm and CAR ≤ 1.25 were considered as cut-off points between successful and subpar groups [12]. In addition, Mahfouz defined P/A LLR > 0.5 as a cut-off between the successful and unsuccessful groups [17]. In our study, the cut-off point for calcification that predicted procedure success was Ca score ≤ 2, which means a calcium score less than moderate, as demonstrated by Soliman et al., who graded leaflet calcification to four grades modified from Anwar et al.[10] with permission as follows: normal=0, mild=1–2, moderate=3–5, and severe ≥6 [21].

Finally, we discovered that these quantitative characteristics might predict PBMV outcomes and added value to the semi-quantitative traditional approaches we used.

Conclusion

Our study revealed that P/A LLR > 0.55, CAR ≤ 1.25, DH > 1.2 cm, and Ca Score ≤ 2 determined by 2-D and 3-D TEE are specific and sensitive predictors of PBMV success in a quantitative way, aiding the traditional scoring methods in selecting the appropriate patients for the procedure.

The study’s weaknesses were a limited sample size, the lack of multicenter participation, and a short follow-up time.

Statement on ethical issues

Research involving people and/or animals is in full compliance with current national and international ethical standards.

Conflict of interest

None declared.

Author contributions

The authors read the ICMJE criteria for authorship and approved the final manuscript

References

Nutritional and biological value of natural-bio shoots mung bean “Mungoltin”.
Food and biological values

Javokhir B. Khayitov*, Guli I. Shaikhova, Dilshod D. Achilov, Munira J. Allaeva

Tashkent Medical Academy, 100109, Uzbekistan, Tashkent, Fara-bi 2

*Corresponding author: javohirhayitov@mail.ru

Abstract
Based on the results of our own research, examination of scientific dossier materials and reference literature data, it was established that dry powder Mungoltin made from the shoots of mung beans produced by Oriona-Scorpion LLC (Uzbekistan) contains a sufficient amount of protein, minerals, vitamins and dietary fiber, does not have a negative impact on the health status of experimental animals and does not result in functional and material cumulation. Acute systemic toxicity testing with intragastric administration of Mungoltin was carried out in 18 adult white male rats. Animals were divided into 4 groups. The animals of the first group received a nutrition dose of 5000 mg/kg; the white rats of the second group were administered with a dose of 7500 mg/kg, and the rodents in the third group were given a dose of 10000 mg/kg, respectively. The animals in group 4 (the reference group) received distilled water. Upon a prolonged intragastric exposure to Mungoltin, no changes in biochemical parameters were detected. The activity indicators of alkaline phosphatase, trans-aminase enzymes and total protein in the blood serum did not differ significantly from those found in the reference group. Therefore, using Mungoltin will not cause a cytotoxic effect in relation to normal highly proliferating cells in an organism. The results of histomorphological studies of tissues of internal organs upon intragastric administration of Mungoltin within 30 days confirm the absence of toxic effects. According to toxicity parameters under the conditions of the above acute experiments, Mungoltin can be attributed to class 5 practically as a non-toxic substance.

Keywords
Natural-bio-shoots mung bean Mungoltin; Cereal rice, Nutritional and biological values, Comparative assessment, Toxicology

The problems of maintaining and strengthening human health, increasing human life expectancy have always been found and remain on the agenda among the most important and pressing issues in medicine and biology. Currently, the factor of healthy nutrition is recognized as one of the key components in the context of the WHO general strategy, according to which 60% of the causes of mortality are directly related to nutrition problems [13–17]. In addition, it is shown that 80% of adverse environmental factors affect the human body through food and water. It is the nutrients that are transformed during the digestion process into structural and energy substrates, provide for the physical and mental performance and finally determine the duration and quality of life. At the same time, significant changes in the lifestyle of people in recent decades have caused changes in the specific features of diseases, the predominance of growth in nutrition-al-dependent, i.e. food related diseases. Nutrition is a powerful lever for the implementation of the demographic policy of every state, the purpose of which is to maintain the health of the nation [3, 4, 18].

The basis of all life on the Earth is protein. Various forms of proteins take part in all processes occurring in living organisms. In the human body, proteins are responsible for constructing muscles, ligaments, tendons, all organs and glands, hair, nails; proteins are an integral part of all biological fluids and bones. Enzymes and hormones which catalyze and regulate all processes in the body are also proteins [5]. Lack of protein in a diet entails a delay in the overall development of children, their intelligence, and a decrease in immunity. A person with a weakened immune system does not resist infectious diseases, reduces the quality of life and, as a result, it shortens the life span [19].

One of the real possibilities of filling the deficiency of protein, minerals and vitamins is the use of nutritional food Mungoltin made from natural shoots of
mung beans, produced by ORION-SCORPIO LLC. In the Republic of Uzbekistan; unfortunately, the Mungoltin powder made from the sprouts of the sprouted mung beans cannot be found in food industries, despite the high protein content therein and its excellent functional characteristics [17].

Mung beans are an excellent source of protein and high energy. The Mungoltin powder from sprinkled mung beans comes directly from the nutrient-rich mung bean sprouts, containing a large amount of vitamins, minerals, antioxidants and enzymes. It is known that the mung beans are considered to be a valuable source of vitamin A, E, as well as many rare B vitamins. It is also a low glycemic product. During the first few days of germination, the sprout is saturated with potential energy: it is the source of life necessary for the seed growth. The sprout grows and ripens, and concentrated energy increases. The mung beans germinate over a short period of time up to 3.8 cm and can be harvested at the proper time, when they reach the maximum level of their nutritional value. At this point, in the sprout mung bean vitamins and minerals, as well as important phyto-chemical elements, are of the highest efficacy. After young shoots reach this optimal level of their biological development, they are harvested and dried at low temperatures for 8 hours. Drying at low temperatures is extremely important: living enzymes in the sprouts remain alive. At the end of the drying process, the sprouts are carefully crushed into a fine powder. The applicable specifications TU-6425280147-01: 2015 approved by the Ministry of Health of the Republic of Uzbekistan and an applicable international standard have been developed and introduced for this product [17].

As we have already noted, in most papers it is shown that Mungoltin made from the bio-shoots of mung beans is a unique source of high-quality protein. Its average content therein is 12%. The biological value of Mungoltin made from the natural bio-shoots of mung beans is twice as high as that of other plant proteins, and approaches that of the animal-type proteins.

Rice groats have been used by humans for a long time, and it remains a must in every kitchen. Rice groats of white color with a yellowish tint contain about a third of gluten. Nutritional and energetic value of bio-shoots of mung beans and rice groats differs. Therefore, their separate study [9] is reasonable.

The aim of this work is to study the nutritional and biological value of Mungoltin made from natural bio-shoots of mung beans in order to find the most rational ways to use them in human nutrition.

Materials and research methods. The materials for the study cover natural product Mungoltin made from bio-shoots of mung beans as compared with rice groats.

Our studies used the following testing methods: organoleptic, physico-chemical, microbiological testing. In the study of the natural Mungoltin made from bio-shoots of mung beans, we have applied the reference data on the chemical composition of rice groats widely used by the population.

Our studies were conducted at the Department of Hygiene of Children, Adolescents and Food Hygiene at the Tashkent Medical Academy (TMA), Inter-University Research Laboratory (IRL) at TMA and the Test Center of the Institute of Plant Substances named after Academician S.A. Yunusova of the Republic of Uzbekistan.

With the physical characteristics of the studied samples, Mungoltin produced from bio-shoots of mung beans and rice groats, the following indicators were studied:

- humidity, acidity, gluten (15113.4-91, 15113.5-91, 202239-91).

The chemical characteristics of the samples were determined by the following indicators:

- crude protein - by the Kjeldahl method (interstate standard 0846-91);
- total lipids - by the Rushkovsky method (interstate standard 0846-91) with the Soxhlet equipment;
- ash - after burning in a muffle furnace (according to the procedure by A.P. Ermakov, 1972);

Our experimental studies of possible toxic properties consisted of the following stages: the study of the general toxic effect produced Mungoltin made from bio-shoots of mung beans with an assessment of the possible irritating effect on the mucous membranes, as well as a possible cumulative effect thereof; the study of possible allergenic activity and effects made on the central nervous system (CNS) [5, 6, 7].

For the experiment, aqueous solutions (50%) in volumes of 0.3–5 ml per animal were prepared from samples of the natural bio-shoots of mung beans.

The rodents received the same dose in mg per kg of body weight as the object of study within the hours of their observation (16-20 hours). The animals were fed 3 hours after taking the dose. The diet was balanced in protein, fat and carbohydrate, scheduled for laboratory animals.
The effect by Mungoltin from bio-shoots of mung beans and that by rice cereal produced on the central nervous system was evaluated by motor activity, with testing “the number of crossed squares” and assessing the “mink” reflex.

Acute experiments for assessing toxicology with intragastric administration of Mungoltin were carried out in 18 adult white male rats. The animals were divided into 4 groups. The animals of the first group received a dose of 5000 mg/kg; the white rats of the second group were fed with a dose of 7500 mg/kg, and the rodents of the third group were given a dose of 10000 mg/kg. The animals of group 4 (the reference group) received distilled water.

The white rats received 5000, 7500 and 10000 mg/kg per body weight. The animals were fed 3 hours after dosing. The diet was balanced in protein, fat and carbohydrate content, according to a special diet for laboratory animals.

Under the experimental conditions, the acute toxicity of the studied bio-shoots was assessed in 24 white outbred rats with a single intragastric administration of each form of the substance at doses of 5000, 7500 and 10000 mg/kg, respectively. The introduction of the mung bean shoots at higher doses was technically impossible due to the physiologically limited amount of the substance administration into the stomach of the rats.

The maximum tested dose of the substance in experimental animals was 13 times higher than the recommended technological dose, and the reference animals received an equivalent amount of distilled water.

The effect of mung-bean-seedlings-based product Mungoltin on the mucous membranes of the eyes was tested in 2 rabbits. A single inoculation of 0.05 ml of an aqueous suspension of the powder into the conjunctival sac of the left eye of the rabbits was carried out, the right eye served as the reference.

The ability to cumulate the food additive was studied by the Lim subchronic toxicity method under the conditions of multiple intragastric administration to white rats. The experiments were conducted in white rats weighing 158.8 g. Mungoltin was given intragastrically daily for 30 days at an initial dose of 100 mg per kg, followed by an increase of 1.5 times every 5 days. The selected dose approximately corresponds to the average consumption of the mixture in one serving of powder. The reference animals were injected with distilled water in an equivalent volume. In the course of the studies, such indicators of the functional state of animals as survival, general condition, animal activity, dynamics of body weight, morphological composition of peripheral blood and biochemical blood parameters were monitored. [8]

The allergenic effect of the test powder was evaluated by a single intradermal injection of 0.02 ml of solutions of each pharmacological form diluted with physiological saline solution with a concentration of 50% using a tuberculin syringe into the outer surface of the guinea pig ear (6 experimental guinea pigs in the group and 6 in the reference one). The animals were injected with 0.02 ml of the physiological saline for comparison. Sensitization was assessed 12–14 days after the injection of the solution.

All survived animals were decapitated at the end of the study and properly disposed after pathomorphological studies. No organ or tissue has been used for other purposes.

All data obtained during the study was subjected to statistical processing with a Pentium-IV personal computer using the Microsoft OfficeExcel-2003 software package, including the use of built-in statistical processing functions. We used methods of variational parametric and nonparametric statistics, with the calculation of the arithmetic mean of the studied indicator (M), standard deviation (G), standard error of the mean (m), relative values (frequency,%). The statistical significance of the measurements, when comparing the average values, was determined by the Student criterion (t), with the calculation of the probability of error (P) when checking the normality of the distribution (by the excess criterion) and the equality of the general variances (F – Fisher’s test). For statistically significant changes, the confidence level was taken to be P <0.05 [2].

Research results and discussion

We have studied the comparative assessment of the nutritional and biological value of rice cereals and natural bio-shoots of mung beans. Natural bio-shoots of mung beans are intended for the food purposes and are obtained, as indicated above, by grinding mung beans.

To conduct our research, we selected a batch of Mungoltin made from bio-shoots of mung beans and rice cereals, organoleptic, and physical & chemical tests were carried out. A comparison was made with rice cereal.
Organoleptic indicators and such significant physico-chemical parameters as moisture, fat, protein, fiber, acidity, ash, amount and quality of gluten, whiteness, grinding size, drop number, infection, contamination and content of metallomagnetic impurities were determined.

The results obtained by testing of organoleptic and physical & chemical characteristics are listed in Tables 1 and 2 herein.

Table 1
Organoleptic indicators of the quality of flour

<table>
<thead>
<tr>
<th>The name of indicators</th>
<th>Tested types of flour</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bio-shoots of mung beans</td>
<td>Rice groats</td>
</tr>
<tr>
<td>Color</td>
<td>Beige colour</td>
<td>Creamy white</td>
</tr>
<tr>
<td>Smell</td>
<td>Bean characteristic, odor-free</td>
<td>Peculiar to rice cereal, without extraneous odors, not musty, not moldy</td>
</tr>
<tr>
<td>Taste</td>
<td>Inherent, without specific bean smell, bitterness, sour and other extraneous smacks</td>
<td>Peculiar to rice cereal, without extraneous flavors, not sour, not bitter</td>
</tr>
<tr>
<td>Mineral impurities</td>
<td>When masticating, the bio shoots of mung beans Mungoltin, moistened with water, no crunch detected</td>
<td>When chewing rice flour moistened with water, there was no crunch</td>
</tr>
</tbody>
</table>

Table 2
Physical & chemical quality indicators of flour grades

<table>
<thead>
<tr>
<th>The name of indicators</th>
<th>Grade of flour</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bio-shoots of mung beans Mungoltin</td>
<td>Rice groats</td>
</tr>
<tr>
<td>Moisture content, %</td>
<td>6.4±0.51</td>
<td>9.8±1.02</td>
</tr>
<tr>
<td>Fat, %, per dry substance</td>
<td>8.0±0.91</td>
<td>1.57±0.3</td>
</tr>
<tr>
<td>Dry Ash Content</td>
<td>3.43±0.35</td>
<td>0.66±0.08</td>
</tr>
<tr>
<td>Crude fiber, in %, referred to dry matter</td>
<td>8.2±0.84</td>
<td>0.29±0.03</td>
</tr>
<tr>
<td>Crude protein, % dry matter</td>
<td>12.0±2.03</td>
<td>6.92±0.72</td>
</tr>
<tr>
<td>Fineness of grinding, %: the residue on a sieve of silk fabric according to interstate standard 4403; passage through a sieve from silk fabric in accordance with interstate standard 4403</td>
<td>2±0.21 (No. 25)</td>
<td>3±0.29 (No. 43)</td>
</tr>
<tr>
<td>Acidity, °H</td>
<td>20.0±2.35</td>
<td>2.5±0.19</td>
</tr>
<tr>
<td>Metallomagnetic impurity, mg in 1 kg</td>
<td>Traces</td>
<td>Traces</td>
</tr>
<tr>
<td>Pest infestation</td>
<td>Not detected</td>
<td></td>
</tr>
<tr>
<td>Pest pollution</td>
<td>Not detected</td>
<td></td>
</tr>
</tbody>
</table>

The organoleptic characteristics of Mungoltin made from bio-shoots of mung beans are slightly different from those in rice groats, but we have not revealed any negative properties in Mungoltin made from bio-shoots of mung beans.

According to the studied physical and chemical parameters, the above product shows more favorable properties. The powder has a finer grinding, shows a higher ash content, contains more crude fiber and crude protein than rice groats, and it does not demonstrate a greater acidity than rice groats.

As can be seen from the data in Table 3, an analysis of changes in the rats’ body mass shows that throughout the experiment, animals of both groups added weight and the degree of increase in body weight in the experimental: the animals do not differ from the reference. That is, the dynamics of the body weight of animals treated with wheat shoot powder during the entire experiment has no differences with the reference. It is found that the relative mass of the internal organs of the animals of the experimental group differs little from that of the intact group of animals (Table 3 herein).

Table 3
Indicators of the mass of the internal organs of animals treated with Mungoltin under experimental conditions

<table>
<thead>
<tr>
<th>Rat groups</th>
<th>Liver</th>
<th>Kidneys</th>
<th>Spleen</th>
<th>Heart</th>
<th>Adrenal glands</th>
<th>Lungs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference</td>
<td>5.79±0.5</td>
<td>1.35±0.5</td>
<td>0.65±0.1</td>
<td>0.70±0.01</td>
<td>0.017±0.001</td>
<td>1.3±0.1</td>
</tr>
<tr>
<td>Mungoltin</td>
<td>5.71±0.4</td>
<td>1.20±0.6</td>
<td>0.59±0.1</td>
<td>0.72±0.1</td>
<td>0.01±0.001</td>
<td>1.25±0.05</td>
</tr>
</tbody>
</table>

The study of the cytotoxic activity of Mungoltin. For this purpose, samples of rat red bone marrow cells were cultivated.

The obtained rat bone marrow cell samples were divided into 5 groups:
- group I to estimate an effect produced by Mungoltin at a dose of 50 mg/10•10⁶ cells for 60 minutes;
- group II to assess an effect made by Mungoltin at a dose of 25 mg/10•10⁶ cells for 60 minutes;
- group III to estimate an effect produced by Mungoltin at a dose of 10 mg/10•10⁶ cells for 60 minutes;
- group IV to assess an effect made by Mungoltin at a dose of 5 mg/10•10⁶ cells for 60 minutes;
- group V to represent cells without Mungoltin exposure (the reference sample).

The results of assessing the effects made by Mungoltin on the viability of rat red bone marrow cells are presented in Table 4 herein.
Table 4
Cytotoxic activity of Mungoltin against rat red bone marrow cells in vitro

<table>
<thead>
<tr>
<th>No.</th>
<th>Group</th>
<th>Rat Red Bone Marrow Cells</th>
<th>Number of examined cells $10^7$</th>
<th>dead</th>
<th>living</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>abs</td>
<td>%</td>
</tr>
<tr>
<td>1</td>
<td>Mungoltin dose, 50 mg/10$^6$ cells</td>
<td>1.6</td>
<td>5.1x10^5</td>
<td>31.0</td>
<td>69.0</td>
</tr>
<tr>
<td>2</td>
<td>Mungoltin dose, 25 mg/10$^6$ cells</td>
<td>5.0</td>
<td>1.4x10^6</td>
<td>28.0</td>
<td>72.0</td>
</tr>
<tr>
<td>3</td>
<td>Mungoltin dose, 10 mg/10$^6$ cells</td>
<td>9.4</td>
<td>6.0x10^6</td>
<td>63.9</td>
<td>36.1</td>
</tr>
<tr>
<td>4</td>
<td>Mungoltin dose, 5 mg/10$^6$ cells</td>
<td>0.98</td>
<td>2.6x10^5</td>
<td>25.0</td>
<td>75.0</td>
</tr>
<tr>
<td>5</td>
<td>Reference</td>
<td>1.1</td>
<td>2.6x10^5</td>
<td>23.6</td>
<td>76.4</td>
</tr>
</tbody>
</table>

Our studies have shown that Mungoltin at doses of 50 mg/10$^6$, 25 mg/10$^6$, 10 mg/10$^6$ and 5 mg/10$^6$ does not significantly affect the viability of rat red bone marrow cells in vitro. Mungoltin at its highest dose of 50 mg/10$^6$ cells, prepared as semi-finished ice-cream-type product has led to death of 31.0 % of the lymphoid cells, while the similar indicator in the reference group has been recorded to reach 23.6 %, i.e. the resulting difference in the number of dead cells when using the Mungoltin food supplement compared with the reference has been found to be within the statistical error.

Our macroscopic examinations of the internal organs in the rats have shown no change therein. The animals are neat in appearance, the coat is shiny, and there are no alopecia signs. The visible mucous membranes are pale pink, shiny, smooth. The mammary glands in the female rats show no abnormalities on palpation. The genitals in the male rats are found to be properly developed. The tails are noted to be somewhat brownish in color. The alveolar and bronchial system in the lungs has the normal regular structure. Plethora has been estimated as moderate. The blood vessels of the lungs have been found to be moderately full-blooded. The alveoles are filled with air. Edema or inflammation of the lung tissue has not been revealed. The size of the heart is within its normal limits. There is a small amount of blood found in the chambers of the heart. The cardiac muscle is dense and brown in color. The stomach and the small intestine are found to be without any pronounced changes. The stomach is filled with a small amount of solid food. The mucous membrane is shiny, folded and somewhat pink in color. The mucosa of the small intestine is shiny, smooth and pink in color. The size and the shape of the liver show no differences from the reference. The surface of the liver is smooth. The capsule of the liver is thin and transparent. The lobular structure of the liver is preserved. The kidneys are of their normal size and shape, brown in color, dense, with a distinct cortical and medullary substance in the cross-section.

Thus, pathomorphological studies confirm the harmlessness of dry powder Mungoltin from the shoots of mung beans.

Conclusion
The results of our studies allow us to discuss a number of important medical and hygienic aspects related to the study of the nutritional value of dry powder Mungoltin made from the shoots of mung beans and rice grits. Consequently, as a result of the assessment of the organoleptic, physical & chemical and nutritional value, it can be concluded that Mungoltin in the form of the powder made from the shoots of mung beans, containing ample proteins, lipids, minerals, vitamins and dietary fiber, represents a valuable complex of unique essential nutritional factors. Mungoltin made from shoots of mung beans is a natural food product. Mungoltin prepared as a drink enhances the performance of the immune system, improves metabolism, and also increases the nutritional and biological value of the product as a nutritional supplement. Thus, the comparative analysis of the results of the study of dry powder Mungoltin made from the shoots of mung beans as against rice groats reveals significant differences therein.

Based on the results of our own research, our review of scientific data and the relevant reference literature, it has been found that dry powder Mungoltin made from the shoots of mung beans, produced by ORIONA-SCORPION LLC (Uzbekistan), does not adversely affect the health of experimental animals and does not result in functional or material cumulation. According to the tested toxicity parameters under the conditions of acute experiments, Mungoltin can be attributed to class 5, and it can be considered practically as a non-toxic substance.

With prolonged intragastric administration of Mungoltin no changes in the tested biochemical pa-
rameters have been detected. The activity indicators of alkaline phosphatase, trans-aminase enzymes and total protein in the blood serum have shown no significant differences from the reference values.

The results of studying of possible cytotoxic effect in vitro show that Mungoltin at its doses of 50 mg/10•10^6, 25 mg/10•10^6, 10 mg/10•10^6 and 5 mg/10•10^6 does not significantly affect the viability of red cells rat bone marrow in vitro.

Therefore, the use of Mungoltin is free of any cytotoxic effects in relation to normal highly proliferating body cells.

The results of our histomorphological studies of tissues of the internal organs with intragastric administration of Mungoltin within 30 days confirm the absence of toxic effects.

Thus, the conducted toxicological studies allow us to draw a conclusion about the biomedical safety of dry powder Mungoltin made from the shoots of mung beans, produced by ORIONA-SCORPION LLC.

**Recommendations**

Using of Mungoltin made from mung bean bio-shoots as a product for the population and as an organic, natural, energy- and protein-containing drink improves the performance of the digestive system, removes toxins and harmful substances from the body and strengthens the immune system.

**Statement on ethical issues**

Research involving people and/or animals is in full compliance with current national and international ethical standards.

**Conflict of interest**

None declared.

**Author contributions**

The authors read the ICMJE criteria for authorship and approved the final manuscript.

**References**

7. San R and N 0283-10 of 02/05/10 «Hygienic requirements for food safety».Tashkent: 2010: 48 p. [in Russian]


Predictors of residual coronary artery disease after PPCI in diabetic patients with STEMI

Bassem Zarif*

Department Cardiology, National Heart Institute (NHI), Cairo, Egypt.

*Corresponding author:
+201223950548
bassemzarif@gmail.com/

Abstract

Objective: To describe the patients’ characteristics and risk factors of diabetic and non-diabetic patients presenting with ST-elevation myocardial infarction (STEMI) in relation to residual SYNTAX score after PPCI (primary PCI).

Material and Method: Prospective observational study using data from all comers in NHI with STEMI and underwent PPCI over one month.

Results: The present study consisted of 290 patients with ST-elevation myocardial infarction over one month period. About 50% of the patients with diabetes were more often female, with a greater prevalence of hypertension and dyslipidemia. In the diabetic group, the eGFR <60 ml/kg/min was associated with more residual CAD after PPCI and a higher residual SYNTAX score.

Conclusion: eGFR is correlated with the presence of MVD in diabetic patients and with a higher residual syntax, which reflects the severity of disease in diabetic patients with nephropathy. That observation was not existing in a diabetic with normal eGFR or in non-diabetics with normal or reduced eGFR.

Keywords
STEMI, Diabetes, eGFR, SYNTAX

Background

Coronary artery disease is the leading cause of morbidity and mortality in diabetes mellitus [1]. Patients with diabetes who developed an acute coronary syndrome appeared to sustain worse outcomes than those without diabetes. The poor prognosis associated with diabetes after acute myocardial infarction has been observed in several studies [2] despite adjustment for age, sex, additional comorbidities, and coronary risk factors. These differences may be related to the severity and extent of coronary heart disease in diabetic patients.

According to the available literature, the prevalence of a significant multivessel coronary disease among patients with STEMI varies from 30% to 60% [3].

The natural history studies of STEMI that multiple plaque ruptures can occur distant from the site of the culprit lesion throughout the coronary tree, suggesting a dynamic process of plaque instability which would be associated with worse prognosis and a higher risk of ischemic recurrences [4].

Glomerular filtration rate (GFR) is the best overall index of kidney function. Normal GFR varies according to age, sex, and body size and declines with age. Chronic kidney disease (CKD) affects a substantial minority of people with type 2 diabetes (T2D). Analysis of US National Health and Nutrition Examination Survey (NHANES) datasets from 2007 through 2012 showed Stage 3 or worse disease (estimated glomerular filtration rate [eGFR] <60 mL/min per 1.73 m²) in nearly one in five patients [5].

More recently, the significant impact of chronic kidney disease (CKD) on cardiovascular risk has been increasingly recognized. Patients with CKD are far more likely to die, predominantly from CVD, than to progress to end-stage renal disease (ESRD) [6].

Residual SYNTAX Score (RSS) was derived from the SYNTAX score to quantify the burden of residual coronary artery disease after percutaneous coronary intervention (PCI) or coronary artery bypass graft (CABG) and has been validated as an independent predictor for clinical adverse events [7].

Aim of work

The purpose of the present study was to describe differences in presenting characteristics of diabetic and non-diabetic patients with ST-elevation myocardial infarction and the relation with residual syntax score after PPCI using data from all comers to NHI over one month period.
Material and Method

Study design
This is a prospective observational study for all STEMI comers present to National Heart Institute for one month and underwent PPCI.

Study population
Our cohort recruited 290 patients who presented over 30 days to NHI with STEMI. Between 1-30 September 2021. Information about patients’ demographic, presenting symptoms, management practices coronary syndrome was recorded. The registry classified the patients into two groups, diabetics, and non-diabetics.

The present study included ST-elevation myocardial infarction, which is determined by chest pain and/or symptoms felt to be consistent with cardiac ischemia and elevated biochemical markers of myocardial necrosis, either total creatine phosphokinase or creatine kinase MB fraction > two times the upper limit of the hospital’s standard range and/ or positive troponin I or T results (if performed) with accompanying electrocardiographic changes fitting criteria for ST-segment elevation or new LBBB. Diabetes is diagnosed when the patient’s fasting plasma glucose is 126 mg/dL or higher at least two times or has a history of diabetes.

eGFR calculated using: The National Kidney Foundation recommends using the CKD-EPI Creatinine Equation (2021) to estimate GFR [8]. Residual SYNTAX Score (RSS) was used to estimate residual CAD after PPCI to the culprit lesion. It was calculated after the final angiogram using the online SYNTAX calculator [9], excluding the culprit lesion.

Statistical analysis
Categorical data were summarized in frequencies and percentages. Continuous variables were reported as mean ± SD or median and 25th and 75th percentiles. Differences in baseline characteristics, presentation, treatment practices among the comparison groups were examined using the Chi-Square test and t-test for categorical and continuous variables, respectively. Mann-Whitney U-test was used for comparing time to admission and length of stay between two groups. The significance of the difference was defined as a two-tailed p-value < 0.05. Logistic multivariable regression analysis by the SPSS program was used to examine differences in the residual SYNTAX for diabetes and other potentially confounding prognostic factors from baseline characteristics, clinical presentation.

<table>
<thead>
<tr>
<th></th>
<th>Group</th>
<th>N</th>
<th>Mean ± SD</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>Non-Diabetics</td>
<td>176</td>
<td>58.420 ± 10.970</td>
<td>&lt; .001</td>
</tr>
<tr>
<td></td>
<td>Diabetics</td>
<td>114</td>
<td>54.956 ± 9.357</td>
<td></td>
</tr>
<tr>
<td>Female Sex</td>
<td>Non-Diabetics</td>
<td>38/176</td>
<td>0.784 ± 0.413</td>
<td>0.028</td>
</tr>
<tr>
<td></td>
<td>Diabetics</td>
<td>38/114</td>
<td>0.664 ± 0.475</td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>Non-Diabetics</td>
<td>176</td>
<td>28.055 ± 3.876</td>
<td>0.009</td>
</tr>
<tr>
<td></td>
<td>Diabetics</td>
<td>114</td>
<td>29.338 ± 4.103</td>
<td></td>
</tr>
<tr>
<td>HTN</td>
<td>Non-Diabetics</td>
<td>176</td>
<td>0.239 ± 0.427</td>
<td>&lt; .001</td>
</tr>
<tr>
<td></td>
<td>Diabetics</td>
<td>114</td>
<td>0.673 ± 0.471</td>
<td></td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>Non-Diabetics</td>
<td>176</td>
<td>0.153 ± 0.361</td>
<td>0.009</td>
</tr>
<tr>
<td></td>
<td>Diabetics</td>
<td>114</td>
<td>0.372 ± 0.485</td>
<td></td>
</tr>
<tr>
<td>Smoking</td>
<td>Non-Diabetics</td>
<td>176</td>
<td>0.602 ± 0.491</td>
<td>&lt; .001</td>
</tr>
<tr>
<td></td>
<td>Diabetics</td>
<td>114</td>
<td>0.389 ± 0.490</td>
<td></td>
</tr>
<tr>
<td>eGFR (mL/min per 1.73 m²)</td>
<td>Non-Diabetics</td>
<td>176</td>
<td>82.490 ± 26.479</td>
<td>&lt; .001</td>
</tr>
<tr>
<td></td>
<td>Diabetics</td>
<td>114</td>
<td>68.040 ± 26.228</td>
<td></td>
</tr>
<tr>
<td>Residual Syntax score &gt;0</td>
<td>Non-Diabetics</td>
<td>33/176</td>
<td>6.045 ± 9.302</td>
<td>0.015</td>
</tr>
<tr>
<td></td>
<td>Diabetics</td>
<td>40/114</td>
<td>9.274 ± 11.860</td>
<td></td>
</tr>
</tbody>
</table>

Baseline characteristics
The present study consisted of 290 patients with elevation myocardial infarction. 176 were non-diabetics (60.6%), while 114 were diabetics. Diabetic patients were younger in average age (54 vs. 58 years) (figure 1). Patients with diabetes were more likely to be women (34% vs. 21%) (figure 2), and they were more likely to have a higher BMI (Figure 3), hypertension (figure 4), and dyslipidemia (Figure 5). They were less likely to be current cigarette smokers (figure 6). eGFR was less in diabetics (68 mL/min per 1.73 m² vs 82 mL/min per 1.73 m²) (Figure 7). Both groups’ management strategies were similar, and the whole study population received PPCI to the culprit-only lesion.
Figure 1. Mean age distribution between both groups; green plot represents non-diabetics and orange represent diabetics

Figure 2. Gender distribution between both groups; green plot represents non-diabetics and orange represent diabetics

Figure 3. BMI distribution between both groups; green plot represents non-diabetics and orange represent diabetics

Figure 4. HTN distribution between both groups; green plot represents non-diabetics and orange represent diabetics

Figure 5. Dyslipidemia distribution between both groups; green plot represents non-diabetics and orange represent diabetics

Figure 6. Smoking distribution between both groups; green plot represents non-diabetics and orange represent diabetics

Figure 7. eGFR distribution between both groups; green plot represents non-diabetics and orange represent diabetics
Predictors of residual SYNTAX score

217 patients have a residual SYNTAX score of zero (75%), while 73 (25%) have residual syntax more than zero, which is considered a multivessel disease (MVD). Diabetic subgroup 35% (40 patients) have MVD while 18% (33 patients) of the non-diabetic subgroup have MVD.

Then the association between residual syntax score groups and every other measured variable was tested (Univariate analysis). Diabetes, hypertension, and dyslipidemia showed no significant correlation with residual SYNTAX (p=0.7).

Correlation between eGFR and residual SYNTAX (Figure 8):
• Diabetic patients with eGFR<60 mL/min per 1.73 m² was found in 54 patient of the diabetic subgroup. Multivessel disease 30 (57%) patients average residual syntax above zero with average residual SYNTAX of 25. While 60 diabetic patients have an eGFR >60 mL/min per 1.73 m²; of them, 17 (28%) has MVD, average syntax 22
• Non-diabetic with eGFR<60 mL/min per 1.73 m²: 35 patient; of them (17%) has residual syntax above zero with an average of 18.

Discussion

The presented study found that 40% of ST-elevation myocardial infarctions were diabetes, while approximately one-quarter of patients with ACS had diabetes from the GRACE registry. Diabetic patients in the present study were more often female, more likely to have hypertension and dyslipidemia, less likely to be a smoker, which is consistent with the data available from the GRACE registry. 25% of all study cohort showed MVD, which is less than observed in worldwide registries, which showed an average of 40-60% of all patients with STEMI that showed MVD [10].

Diabetics have a higher possibility to have MVD than non-diabetics which is consistent with the available data [11].

However, the presence of diabetes was not independently correlated with the residual disease after treating the culprit lesion with PPCI. Other baseline characteristics showed no significant correlation with the residual SYNTAX, including gender, hypertension, smoking, and dyslipidemia.

We observed in our study that diabetic patients with eGFR < 60 mL/min per 1.73 m² have a higher residual SYNTAX score than diabetics with normal GFR as well as non-diabetic with normal or reduced eGFR, which might represent a link between Cardiovascular and renal outcomes in diabetic patients.

Before our work, the residual SYNTAX score after PPCI was not tested on randomized data as a potential predictor of recurrent events. However, the patients with MVD presented with STEMI have a worse outcome and a higher rate of recurrent events [12]. More research is needed to evaluate the outcome of this group of patients.

Conclusion

eGFR is correlated with the presence of MVD and with higher residual syntax score in diabetic patients presented with STEMI, which reflect the relation between the severity of CAD and nephropathy in diabetic. That observation was not existing in a diabetic with normal eGFR or in non-diabetics with normal or reduced eGFR.

Calculation of eGFR in all diabetic patients with STEMI should be encouraged and included in the baseline assessment of the patient’s risk profile.

Our observation generates a hypothesis about the missing link between micro and macrovascular complications in diabetes.

Further research is needed regarding cardiovascular outcomes and possible interventions that may improve renal and vascular outcomes in diabetic patients with nephropathy after STEMI.

Acknowledgment

We thank the CCU team at NHI for their help in data collection.

Statement of ethics

We declare that the research complies with the guidelines for human studies following the World
Medical Association Declaration of Helsinki. The ethics committee approved the study protocol of the general organization for teaching hospitals and institutes (GOTHI). Number IHC00019, date 14/7/2021. A written Informed Consent was obtained from all participants to participate in the study.

The authors have no conflicts of interest to declare.

No funding

Authors’ contributions
EC wrote the manuscript. BZ and KL wrote the study protocol and supervised data collection and analysis, and all authors reviewed the final manuscript.

Data availability
All data generated or analyzed during this study are included in this article [and/or] its supplementary material files. Further inquiries can be directed to the corresponding author.

References
Development of modern technology for obtaining tinctures with sedative effect

Holida M. Yunusova*, Zilola V. Turdieva, Nargiza B. Ilkhamova

Tashkent Pharmaceutical Institute, 100015, Uzbekistan, Tashkent, 45 Aybek Street

*Corresponding author:
holida_222@mail.ru

Abstract

The aim of this work was to experimentally select the optimal conditions for obtaining a tincture with an effective sedative effect consisting of a mixture of common motherwort grass, medicinal lemon balm and nettle leaves in a ratio of (1:1:1) and the development of quality criteria for the finished product. The influence of the concentration of the extractant, the ratio of raw materials to extractant, the multiplicity of extraction and the mode of infusion with the use of auxiliary technologies (ultrasound treatment) on the yield of biologically active substances from the raw materials was studied. It has been established that the most acceptable way to obtain this tincture is maceration using ultrasound. This method allows us to obtain a tincture with a high yield of flavonoids with the minimum time used.

Keywords

Tincture, Maceration, Medicinal plant, Flavonoids, Extractant

We cannot underestimate the importance of sedatives in our time rich both in negative stresses like difficult psychological situations, family and industrial conflicts and positive stresses like immatriculation, promotion, the birth of a child etc. Drugs of the sedative group (from Lat. sedatio - sedation) have a regulating effect on the functions of the central nervous system, enhancing the processes of inhibition or lowering the processes of arousal, eliminating the effects of stress. Despite the presence of modern tranquilizing agents, which also have a sedative effect, sedatives are widely used in medical practice under various neurotic conditions, mild sleep disorders, neurogenic diseases (gastric ulcer and duodenal ulcer, hypertension, coronary heart disease), neurocircular dystonia, menopausal syndrome. It is important that, unlike "classic" tranquilizers, they do not pose a danger associated with the development of addictive phenomena, mental and physical dependence. According to WHO, up to 80% of the world’s population prefer to be treated with natural herbal remedies. Herbal preparations are preferred due to their low toxicity, the possibility of long-term use without significant side effects, a wide range of pharmacological activity, relatively low cost and ease in production [1, 2, 3].

Prominent representatives of this group of drugs are well-known medicinal plants such as motherwort, melissa officinalis and nettle dioecious.

Common motherwort (Leonorus cardiaca) is a plant belonging to the family of Lamiaceae, which has a pronounced sedative effect. Motherwort preparations are prescribed against increased nervous excitability, cardiovascular neuroses, sleep disorders, neurocircular dystonia, at the early stages of hypertension, after flu and some other infectious diseases accompanied by cardiosclerosis, angina pectoris, myocarditis and myocardystrophy, in case of heart defects, basal disease, hypersthenic neurasthenia, hysteria, epilepsy, algodismenorrhea and menopausal syndrome. In dermatology, it is used in treatment of neurodermatitis, eczema, itching, lichen planus and psoriasis mainly as a sedative drug, in its also administered in pediatric practice and in case of microbial eczema with functional disorders of the nervous system. Motherwort grass is a part of the sedative herbal tea blends.

Melissa officinalis is a perennial herbaceous plant containing essential oil, a species of the genus Melissa of the Lamiaceae family. Indications for the use of Melissa preparations are neuroses, heart diseases, acute and chronic gastrointestinal diseases, inflammatory diseases of the respiratory system, eczema, dermatitis, some gynecologic diseases, pregnancy toxicosis, immunodeficiency. The leaves and tops of the shoots of this plant can be applied externally in case of tooth pain, rheumatism and hematomas.

Nettle dioecious (Urtica dioica) is a perennial herbaceous plant of the Urticaceae family. Nettle prepa-
rations are used to solve some problems with copious menstrual bleeding disorders, in case of non-copious bleeding in the post-coagulation period of treatment of cervical erosion, in case of menopausal bleeding, subserous fibromyomas, in case of some abnormal postpartum events like involution of the uterus. Decoction from the leaves is taken in treatment of diseases of the liver, the biliary tract and in case of helminthic invasion. In dental practice it can be administered to treat periodontitis and gingivostomatitis [4, 5].

The aim of our study is to develop a method for obtaining a tincture with a sedative effect, conventionally called “Biosedation”, consisting of a mixture of common motherwort grass, medicinal lemon balm and nettle leaves in a ratio (1:1:1).

For the study, grass and leaves of plants collected in the period May–July in the territory of the Tashkent region were used. Drying of plant material in one layer was carried out in the natural way at a room temperature in a ventilated room with periodic turning over of the materials. As a result from using the method of natural drying, intact dried leaves of the plant were obtained.

The next step was to select the optimal extractant. Our experiment was carried out with three samples selected on the basis of the available reference literature data. We selected ethyl alcohol solutions of various concentrations as samples. Extraction was carried out from the weights of the same sample of raw materials, under the same conditions (at a room temperature, for 3 hours); the ratio of raw materials to extractant was 1:10. The produced tinctures were clear liquids, light brown in color, with a characteristic aromatic odor. The yield of flavonoids from plant raw materials was determined by spectrophotometry. As a result of the experiment, 70% ethyl alcohol was chosen as the optimal extractant, since the maximum yield of flavonoids was extracted by this particular extractant (see Figure 1 herein).

Quantitative assessment of the number of flavonoids. Our assessment was carried out by the spectrophotometric method. 2.0 ml of the drug was placed in a measuring flask with a capacity of 25 ml, then 5 ml of a 2% alcohol solution of aluminum chloride was added, placed for 3 minutes in a boiling water bath, quickly cooled and made up with the 70% alcohol solution to reach the required measuring mark.

The optical density of the obtained solution was measured with a spectrophotometer at a wavelength of 409 nm in a cuvette with a layer thickness of 10 mm, using a solution consisting of 2.5 ml of medicine as the reference solution, which was placed in a measuring flask with a capacity of 25 ml with adjusting the volume with the 70% alcohol solution to the required measuring mark.

The optical density of a solution containing 1 ml of a solution of SOVS rutin treated analogically to the test solution was measured in parallel, placed in a measuring flask with a capacity of 25 ml with adjusting the volume with the 70% alcohol solution to the required measuring mark, using a solution consisting of 1 ml of a solution of SOVS rutin as the reference, which was placed in a measuring flask with a capacity of 25 ml and the volume of the solution adjusted with 70% alcohol to the designed mark.

The content of the number of flavonoids in the preparation (X, g / ml), in terms of rutin, was calculated by the formula:

\[
X = \frac{D_t \times 25 \times a_0 \times P}{D_0 \times 25 \times 100 \times 2.5 \times 100},
\]

where: \(D_t\) is the optical density of the test solution; \(D_0\) is the optical density of the SOVS rutin solution; \(P\) is the content of routine in the SOVS, in %; \(a_0\) is the weight of the suspension of the SOVS rutin, in g.

The number of flavonoids in the preparation, in terms of rutin, should be at least 0.0003g / ml.

Note: Preparation of the solution is rutin.
About 0.05 g of rutin (USP, BP, Eur. Ph., CP, “Sigma” catalog number 125143 or similar quality) (exact weight) pre-dried at 130-135 ° C for 3 hours, dissolved in 50 ml of 70% ethyl alcohol in a 100 ml volumetric flask, was heated in a boiling water bath, cooled to a room temperature, the volume of the solution alcohol is made up to reach the required measuring mark and stirred. The shelf life of the solution is 1 month.

Preparation of 2% alcohol solution of aluminum chloride.
2 g of aluminum chloride was dissolved in 50 ml of 70% alcohol in a measuring flask with a capacity of 100 ml, the volume of the solution is brought to reach the required measuring mark with alcohol and mixed [6, 7, 8].

**Investigation of extraction dynamics.** When extracting plant raw materials, in order to prevent unreasonable costs, it is necessary to study the required duration of the extraction process. Therefore, in order to determine it and establish the time of the phase equilibrium, we have studied the dynamics of the extraction process. There is data available in the reference literature on the study of the dynamics of the process with various extraction methods. However, first of all it depends on the type of raw materials used [5].

Experiments to determine the time of the phase equilibrium were carried out according to the methodology described in our paper. The study of the extraction dynamics has shown that the concentration of flavonoids in the tincture gradually increases and reaches a maximum by 6 days (see Figure 2 herein).

![Figure 2. Effect produced by extraction time on flavonoid yield](image)

**The effect made by the degree of grinding of raw materials on the yield of flavonoids from medicinal plant raw materials.** Of particular importance for the extraction of active substances from plant material is the degree of its grinding. It is known that the greater the fineness, the more complete the diffusion proceeds, which is due to an increase in the contact surface of the phases. To a large extent, the completeness of the extraction of the target substance depends on the degree of grinding of plant raw materials.

When searching for the optimal value of the degree of grinding, we used raw materials which were crushed to various degrees: larger than 10 mm, 3–10 mm, 1–3 mm, and smaller than 1 mm. As expected (see Figure 3 herein), with finer grinding of raw materials, the yield of flavonoids increased. The grinding of raw materials contributes to an increase in the surface area of the phase interface, but at the same time the inadmissibility of slurping with excessively fine grinding should be taken into account. Thus, the yield of active substances when using raw materials with a size greater than 10 mm is 3.8%, when grinding 3–10 mm, reaches 4.5 %, at 1–3 mm we obtain 5.2%, and under 1 mm we can obtain even 5.5%. However, taking into account that the excessively fine grinding of raw materials significantly complicates the filtration of the resulting tincture, and, moreover, the yield of flavonoids from raw materials crushed less than 1 mm varies slightly, we have decided that the grinding degree of 1–3 mm is the best suitable for our purpose.

![Figure 3. Results of studying the degree of grinding of raw materials](image)

**Selection of the ratio of raw materials to extractant.** At the next stage of the study, the optimal ratio of raw materials to extractant was determined to increase the yield of extracted substances. As can be seen from Table 1, the yield of active substances from raw materials is the highest at a ratio of 1:10.

<table>
<thead>
<tr>
<th>The ratio of raw materials and extractant</th>
<th>The degree of extraction of the number of flavonoids, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:5</td>
<td>48.1-54.9</td>
</tr>
<tr>
<td>1:10</td>
<td>87.7-91.2</td>
</tr>
<tr>
<td>1:20</td>
<td>65.5-79.8</td>
</tr>
</tbody>
</table>

**Selection of the extraction method.** The following extraction methods were considered as possible methods for obtaining alcohol extraction from raw materials: maceration, bismaceration, maceration+UST (ultrasound treatment), bismaceration+UST and remaceration +UST.
The maceration method is a recognized extraction method, which is very widely used in the pharmaceutical industry. This method does not require complex equipment; it is a simple technology showing a good yield of a biologically active complex of extractive substances. However, as our analysis of the reference literature shows, this method is losing its popularity due to a number of its disadvantages such as high time- and labor-intensity; possible evaporation of the extractant, an incomplete extraction of BAS, lower yields compared to other extraction methods. In this regard, alternative extraction methods are being developed: remacery, turbo extraction, ultrasonic and microwave extraction, percolation, extraction with liquefied gases and others.

The method of ultrasonic extraction is considered by many authors as a method of intensification in the technology of producing extracts. The spectrum of waves involved in ultrasound is called ultrasonic waves, and the frequencies of these waves are above the sound range (> 20 kHz) and below the microwave frequencies (up to 10 MHz). The process can be organized by direct exposure of ultrasound to the sample using a probe or indirectly in an ultrasound bath through the walls of a vessel containing a sample to be treated. Ultrasonic exposure is based on the principle of acoustic cavitation, which destroys cellular structures. With the increase in ultrasonic power, the number of cavitation bubbles increases, the collapse of which leads to some local temperature and pressure jumps. This process facilitates the disintegration of plant cells [9, 10].

When analyzing the effect produced by ultrasound on the extraction of flavonoids from a mixture of parts of medicinal plants of motherwort, medicinal melissa and nettle leaves, the most optimal extraction method, considering the quantitative content of flavonoids in terms of rutin, is maceration using ultrasound treatment, and the most optimal extractant in this case is 70% ethyl alcohol. According to the obtained results of the influence of the degree of grinding of raw materials on the yield of flavonoids from medicinal plant raw materials, our decision is to utilize grinding degree of 1-3 mm, and the ratio of raw materials to extractant shall be 1:10.

<table>
<thead>
<tr>
<th>Infusion mode</th>
<th>Description</th>
<th>Dry residue, %</th>
<th>Flavonoid content (in terms of rutin), g/ml</th>
<th>Ethanol content, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maceration</td>
<td>Transparent liquid, light brown in color, with a characteristic aromatic odor</td>
<td>1,7</td>
<td>0,0028±0,03</td>
<td>64</td>
</tr>
<tr>
<td>Bismaceration</td>
<td></td>
<td>1,5</td>
<td>0,0028±0,03</td>
<td>63</td>
</tr>
<tr>
<td>Maceration+UST</td>
<td></td>
<td>1,9</td>
<td>0,0003±0,03</td>
<td>89</td>
</tr>
<tr>
<td>Bismaceration+UST</td>
<td></td>
<td>1,4</td>
<td>0,0028±0,01</td>
<td>75</td>
</tr>
<tr>
<td>Remaceration+UST</td>
<td></td>
<td>1,4</td>
<td>0,0028±0,03</td>
<td>68</td>
</tr>
</tbody>
</table>

Conclusions

When developing a method for obtaining a tincture with a sedative effect, consisting of a mixture of common motherwort grass, medicinal melissa and nettle leaves, the most optimal extraction method, considering the quantitative content of flavonoids in terms of rutin, is maceration using ultrasound treatment, and the most optimal extractant in this case is 70% ethyl alcohol. According to the obtained results of the influence of the degree of grinding of raw materials on the yield of flavonoids from medicinal plant raw materials, our decision is to utilize grinding degree of 1-3 mm, and the ratio of raw materials to extractant shall be 1:10.

Statement on ethical issues

Research involving people and/or animals is in full compliance with current national and international ethical standards.

Conflict of interest

None declared.

Author contributions

The authors read the ICMJE criteria for authorship and approved the final manuscript.

References


Pre-angioplasty instantaneous wave-free ratio pullback predicts hemodynamic outcome in diffuse coronary artery disease

Ahmed Youssef Nammour1*, Hisham Mohamed Aboul-Enein1, Mohammed Hamouda1, Ahmed Atef Elhelaly2

1Department of Cardiology, Benha University, Egypt.
2National Heart Institute, Cairo, Egypt.
* Corresponding author:
info@bu.edu.eg

Abstract

Background: Serial stenoses or diffuse vessel narrowing hamper pressure wire-guided management of coronary stenoses. Characterization of functional relevance of individual stenoses or narrowed segments constitutes an unmet need in ischemia-driven percutaneous revascularization.

Aim of the Study: To perform hemodynamic mapping of the entire vessel using pullback technique of a pressure guidewire with continuous instantaneous wave-free ratio (iFR) measurement compared coronary angiography aiming to minimize the procedure, decrease number and length of stents used.

Materials and Methods: This study was conducted on 40 patients presented with diffuse coronary artery disease and undergoing elective PCI. Diagnostic coronary angiography using the routine angiographic projections was done with assessment of non-obstructive coronary lesions by 2D quantitative coronary angiography and iFR pullback measurement.

Results: Percentage of difference between probable sig lesions via pullback technique and No of actual sig lesions for studied group, was (59.5%). The difference was statistically highly significant p=0.0001.

Conclusion: Compared with angiography alone, availability of iFR pullback data significantly decreased the number and length of hemodynamically significant lesions identified for revascularization.

Keywords
Coronary artery disease, Instantaneous wave-free ratio, Physiological lesion assessment, Stenosis

Introduction

The presence of myocardial ischemia is a significant risk factor for an adverse clinical outcome. Revascularization of obstructive coronary lesions that induce ischemia can improve a patient’s functional status and outcome [1].

For stenotic lesions that do not induce ischemia, however, the benefit of revascularization is less clear, and medical therapy alone is likely to be effective [2].

With the introduction of drug-eluting stents (DES), the percentage of patients with multivessel coronary artery disease in whom percutaneous coronary intervention (PCI) is performed has progressively increased [3]. However, in patients with multivessel coronary artery disease (MVD), determining which lesions cause ischemia and deserve stenting can be challenging. Noninvasive stress imaging studies has limited ability to accurately confine ischemia-producing lesions in these patients [4]. Although coronary angiography may underestimates /overestimates a lesion’s functional severity, it is still the standard technique to guide PCI in patients with MVD [5].

Pressure wire technology has revolutionized the treatment of coronary disease, by identifying clinically important stenoses. Physiology provides an objective marker to support intervention by identifying hemodynamically significant lesions. However, most used physiological indices have limited ability to isolate individual lesion significance in vessels with multiple lesions. The prevalence of such diffuse coronary disease is increasing and accurate assessment to guide appropriate therapy is paramount [6, 7].

Instantaneous wave-free ratio (iFR) is a resting index of stenosis severity that is measured without a vasodilator. It is the ratio of distal and proximal pressures over the wave-free period, a specific part of diastole during which coronary flow velocity is naturally at its highest (8). This higher flow velocity allows iFR to
assess higher pressure gradients across stenoses than possible by using the complete cardiac cycle whilst also preserving the key characteristic of constant flow [9]. For the iFR-only strategy, the used a cut-off value of 0.89 below which it is considered a significant stenosis [10].

As a result, iFR has a greater ability to identify small gradients pertinent to the assessment of a diffusely diseased vessel [9,11].

The value of mapping the iFR intensity in diffusely diseased vessels enables identification of any focal areas of disease that may cause the predominant pressure loss, and therefore be targeted for percutaneous intervention. The percentage contribution of pressure loss can be displayed to assist decision-making. The mapping can be displayed in a number of different ways. With co-registration, the pressure wire pullback can become integrated with the angiographic findings to enhance the ease of understanding of the data. 'Dots' representing units of pressure loss can help identify which stenoses are most hemodynamically important. In addition, iFR intensity plotted as a function of distance can give additional information regarding the length over which the pressure drop occurs. This may assist in identifying which lesions in the vessel contribute most to pressure loss and allow operators to estimate the physiological length of a stenosis to help decide between different revascularization strategies [12].

Quantitative coronary angiography (QCA) can be used as another method to help assessment of the coronary lesions. Although intravascular ultrasound (IVUS) currently yields the most accurate measurements of vessel geometry and lesion severity [13]. QCA measurements can be performed on existing standard coronary angiography images without the need for additional time or equipment during the procedure [14].

Resistance to coronary blood flow can be divided into three major components: epicardial arteries, arterioles and microcirculatory resistance arteries and extravascular resistance. There is no measurable pressure drop in the epicardial arteries although in the presence of hemodynamically significant epicardial artery narrowing (more than 50% diameter reduction), the resistance at this level contributes as an important component to the total coronary resistance [15].

**Aim of the work**

To perform hemodynamic mapping of the entire vessel using pullback technique of a pressure guide-wire with continuous instantaneous wave-free ratio (iFR) measurement compared coronary angiography aiming to minimize the procedure, decrease number and length of stents used.

**Patient and methods**

This non randomized controlled trial was conducted at Cardiology Department, Benha University and NHI during the period from August 2020 to August 2021. The study included a group of 40 patients presented with diffuse coronary artery disease and undergoing elective PCI.

**Inclusion Criteria**

Patients undergoing elective PCI with diffuse coronary artery disease.

**Exclusion criteria**

The individuals with the following criteria were excluded from our study; acute MI, simple obstructive and non-obstructive coronary artery lesions, LM lesions, small vessel disease less than 2 mm diameters and myocardial bridge.

**Withdrawal criteria**

The patient has the right to withdraw from the study at any time without any negative consequence on the treatment plane.

All patients were subjected to the following

Complete history taking including age, gender and presence of risk factors for CAD, physical examination, twelve leads ECG, laboratory investigations and echocardiography.

**Coronary angiography and PCI**

Coronary angiography was performed using conventional approaches. Patients underwent a diagnostic coronary angiogram according to the routine clinical practice of the participating center. Angiographic inclusion criteria included the presence of a ≥ 40% stenosis by visual estimate in any major epicardial vessels or any major branch. After angiography, the angiographic images were reviewed, and operators were asked to prospectively document their plans for angioplasty on an electronic case report form. Specifically, operators were required to record the number of angiographically significant lesions and the total lesion length(s) requiring stenting for each patient. This
planning phase, based on visual assessment of angiographic data, was completed before any physiological measurements with iFR pullback.

iFR pullback measurement

Intracoronary nitrates (300 mg) were administered in all cases before pressure wires were introduced. Pressure wire (Prestige guide wire PLUS/Verrata guide wire; Philips/Volcano, Amsterdam, the Netherlands) normalization was performed at the coronary ostia before each recording and before resting pressure wire pullback was performed. iFR was measured in the distal position of the target vessel, followed by an iFR pullback recording along the length of the vessel under resting conditions. Pressure wire pullback was performed in a manual (96.4%) or mechanized manner (3.6%) using Volcano pullback device R100. Pullback speed was 0.5 to 1.0 mm/s and was continued until the pressure sensor reached the left main stem ostium or right coronary ostium. During the pressure wire pullback, regular fluoroscopic recordings of the wire position were performed with accompanying bookmarks on the iFR pullback trace. This allowed operators to determine the trans-stenotic pressure gradient (in iFR units) for each lesion of interest along the entire length of the diseased vessel. In this study, automatic coregistration of the iFR pullback curve with the angiogram was not yet available and thus was not performed.

Post-PCI iFR prediction

According to the aforementioned technique, iFR pullback was used to quantify the iFR gradient at each lesion location of interest along the length of the vessel. The predicted post-PCI iFR (iFRpred) was calculated by summation of the iFR gradient(s) with the distal vessel iFR value. In line with the threshold value used in recent iFR clinical outcome trials (3,4), a post-PCI iFR value ≤ 0.89 was considered suboptimal. Accordingly, operators tailored their PCI approach to achieve a post-PCI iFR value >0.89. At this stage, operators were once more asked to record their interventional strategy with respect to the number and length of lesions to stent based on the addition of iFR pullback to angiogram data.

Post-PCI iFR measurement

Angioplasty was performed as per usual clinical practice using third generation drug-eluting stents, which were all angiographically optimized. Following successful PCI, measurement of the observed post-angioplasty iFR (iFRobs) was performed with the pressure sensor positioned at an identical coronary location as before.

Statistical analysis

All data were collected, tabulated and statistically analyzed using (IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.2015). Quantitative data were expressed as the mean ± SD & median (range), and qualitative data were expressed as & (percentage). Wilcoxon Signed Ranks Test was used to compare between two paired of not normally distributed variable. McNemar test was used to compare between two paired of categorical variable. All tests were two sided.

Results

Table 1
Demographic data for studied group

<table>
<thead>
<tr>
<th>variables</th>
<th>Studied group (n=40)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age per year</td>
<td>56.87±8.63</td>
</tr>
<tr>
<td>Mean ± SD range</td>
<td>38 -74</td>
</tr>
<tr>
<td>sex</td>
<td>24(60%)</td>
</tr>
<tr>
<td>Males</td>
<td>16(40%)</td>
</tr>
<tr>
<td>Females</td>
<td></td>
</tr>
<tr>
<td>HTN</td>
<td>24 (60%)</td>
</tr>
<tr>
<td>DM</td>
<td>26 (65%)</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>25 (62.5%)</td>
</tr>
<tr>
<td>Smoking</td>
<td>22 (55%)</td>
</tr>
</tbody>
</table>

This table shows the mean age of all studied patients was 56.87±8.63 years with range (38-74), 24 of them males (60%), HTN represented 60%, DM 65.0%, dyslipidemia, 62.5% and smokers represented 55%.

Table 2
Difference between No of probable sig lesions via 2D assessment and No of actual sig lesions by IFR among studied group (n=40)

<table>
<thead>
<tr>
<th>No of probable sig lesions by 2D assessment</th>
<th>No of actual sig lesions After iFR</th>
<th>Percentage of difference w</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ±SD</td>
<td>Median Range</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.7±0.93</td>
<td>4 (2-6)</td>
<td>1.5±0.64</td>
<td>1 (1-3)</td>
</tr>
<tr>
<td>(59.5%)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

W = Wilcoxon Signed Ranks Test (HS) p<0.001 highly significant
The above table shows median of lesion via 2D assessment was 4 with range (2-6) higher than detected actually. Percentage of difference between probable sig lesions via 2D assessment and No of actual sig lesions by IFR for studied group, was (59.5%). The difference statistically highly significant p=0.0001.

Table 3
Difference between length expected to be covered mm via 2D assessment and area actually covered after IFR assessment mm among studied group (n. 40)

<table>
<thead>
<tr>
<th>Length expected to be covered (mm)</th>
<th>Length covered after IFR (mm)</th>
<th>Percentage of difference</th>
<th>w</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ±SD</td>
<td>61.3±12.56</td>
<td>36.3±19.25</td>
<td>(40.8%)</td>
<td>5.37</td>
</tr>
<tr>
<td>Median Range</td>
<td>59 (38-92)</td>
<td>37 (0-72)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

W= Wilcoxon Signed Ranks Test       (HS) p<0.001 highly significant

The above table shows median length expected to be covered (mm) via 2D assessment was 59 with range (38–92) higher than detected actually by IFR. Percentage of difference between length expected to be covered (mm) via 2D assessment and area covered (mm) after IFR study for studied group, was (40.8%). The difference statistically highly significant p=0.0001.

Table 4
Comparison between need of stents expected via 2D assessment and stent deployed after IFR study for studied group (n.40)

<table>
<thead>
<tr>
<th>Expected stent needed n. (%)</th>
<th>stent deployed n. (%)</th>
<th>MC p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stent needed</td>
<td>40(100.0)</td>
<td>0.031 (S)</td>
</tr>
<tr>
<td>No Stent needed</td>
<td>0(0.0)</td>
<td>6(15)</td>
</tr>
</tbody>
</table>

MC-Nemar test       (S) p<0.05 significant

This table shows all patients Stent needed via pullback technique. while only 34(85%) actually need stent application the difference statistically significant p<0.05.

Table 5
Comparison between number need of stents expected and number stent deployed after pullback technique for studied group (n.40)

<table>
<thead>
<tr>
<th>number need of stents expected n. (%)</th>
<th>Number stent deployed n. (%)</th>
<th>MC p</th>
</tr>
</thead>
<tbody>
<tr>
<td>one stent</td>
<td></td>
<td></td>
</tr>
<tr>
<td>yes</td>
<td>1 (2.5%)</td>
<td>23(57.5%)</td>
</tr>
<tr>
<td>No</td>
<td>39(97.5%)</td>
<td>17(42.5%)</td>
</tr>
</tbody>
</table>

Regression coefficients(β): represent the mean change in the dependent variable (area covered mm) for one unit of change in the predictor variables. (Std. Error): are the standard errors of the regression coefficients

Significant predictors of area covered mm were length expected to be covered mm by (Wave-Free Ratio Pullback) and patient’s age per years.

Significant predictor of number of affected area was No of probable sig lesions by (Wave-Free Ratio Pullback).
Table 7
Simple linear regression model for prediction of number of affected area among studied patients (n=40):

<table>
<thead>
<tr>
<th>(Constant)</th>
<th>β</th>
<th>Std. Error</th>
<th>t</th>
<th>Sig.</th>
<th>r</th>
<th>R2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.015</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No of probable sig lesions</td>
<td>0.405</td>
<td>0.090</td>
<td>4.52</td>
<td>0.0001</td>
<td>0.591</td>
<td>0.35</td>
</tr>
</tbody>
</table>

Regression coefficients(β): represent the mean change in the dependent variable (area covered mm²) for one unit of change in the predictor variables. (Std. Error): are the standard errors of the regression coefficients. 

R square = 35 % of predictor variance. 

Model ANOVA: F=20.426, p=0.0001

Discussion

In our study we aimed to perform hemodynamic mapping of the entire vessel using pullback technique of a pressure guidewire with continuous instantaneous wave-free ratio (iFR) measurement compared coronary angiography aiming to minimize the procedure, decrease number and length of stents used.

In the current study, the median of lesion via 2D assessment was 4 with range (2-6) higher than detected actually. Percentage of difference between probable sig lesions via 2D assessment and No of actual sig lesions by IFR for studied group, was (59.5%). The difference statistically highly significant p=0.000. In agreement with our study, Kikuta et al. [16] found that the number of coronary lesions determined as hemodynamically significant according to angiographic appearance versus iFR pullback. In 47 of 159 patients (30%) and in 52 of 168 vessels (31%), the number of lesions to treat was changed after iFR pullback measurement. On a per-patient basis, the addition of iFR pullback data decreased the number of lesions identified for revascularization from 1.42±0.05 following angiographic assessment alone to 1.23±0.05 (p = 0.0001 for difference).

In the present study, median length expected to be covered via 2D assessment was 59 with range (38-92) higher than detected actually by IFR. Percentage of difference between length expected to be covered via 2D assessment and area covered (mm²) after IFR study for studied group, was (40.8 %).The difference statistically highly significant p=0.0001. In agreement with our study, Kikuta et al. [16] found that the availability of iFR pullback data decreased the total lesion length identified for revascularization from 31.3±1.3 mm after angiography alone to 26.9±1.3 mm after iFR pullback (p < 0.0001 for difference). Disagreement between total lesion length identified by angiography alone and iFR pullback occurred in 118 patients (74%) in 121 vessels (72%).

Also, all patients needed stent via pullback technique while only 85% actually need stent application via 2D assessment, leading to a fewer stents placed per patient. The difference was statistically significant p<0.05. This came in agreement with Younus, et al., [17], who found that there were significantly fewer hemodynamically significant lesions as assessed by iFR, leading to a fewer stents placed per patient.

The ease of iFR measurement facilitates and encourages the measurement of multiple vessels. And also, iFR-GRADIENT showed there was a significant decrease in the number and length of hemodynamically significant lesions planned for revascularization [18]. Also, Kikuta et al. [16] demonstrates in their multicenter registry study that online iFR pullback performed under resting conditions predicted the physiological outcome of PCI with a high degree of accuracy. They found that Compared with angiography alone, availability of iFR pullback data significantly decreased the number and length of hemodynamically significant lesions identified for revascularization. Overall, revascularization procedural planning was altered in nearly one-third of patients.

Park et al. [19] found that before physical PCI is commenced, iFR pullback data can inform the clinician whether their proposed strategy will improve coronary physiology sufficiently to achieve a physiologically favorable outcome.

The iFR grounds on the concept that at a specific time in diastole – the so-called wave-free period – intracoronary pressure and flow decline together in a linear fashion, whereas microvascular resistance remains more stable and significantly lower than the rest of cardiac cycle. Therefore, over this period, the pressure gradient across coronary stenosis can be measured obviating generating hyperemia through adenosine infusion. Another advantage of iFR is the ability to individually assess lesions severity in the context of diffuse vessel disease. Specifically, by using the coregistration of the iFR pullback trace and the coronary angiogram (ie, plotting measured values directly over angiographic views), iFR is able to detect lesion-specific pressure drop along the whole length of the vessel and differentiate focal from diffuse coronary disease. This allows the cardiologist to (1) prop-
erly identify which lesion/s should be treated (if any), (2) accurately predict to what extent coronary physiology will improve after PCI per each lesion, and (3) confidently decide the number, length, and position of stents to be used to pursue a successful procedure (20).

iFR showed excellent diagnostic test characteristics when compared with other invasive and non-invasive measures of stenosis severity and myocardial ischaemia [21].

In the present study, significant predictors of area covered mm were length expected to be covered mm by (Wave-Free Ratio Pullback) and patient's age per years. Kikuta et al. [16] found that the only univariate and multivariate predictor identified for the difference between both groups was iFR pullback measurement in culprit vessels in patients with acute coronary syndrome (ACS). Age, sex, diabetes mellitus, hypertension, hyperlipidemia, creatinine, smoker, pre-PCI iFR, and number of lesions were not significant predictors of the difference between both groups. Jeremias, [22] found that the available clinical evidence strongly supports the current practice of an ischemia-guided revascularization strategy, in which lesions with abnormal invasive physiology benefit from revascularization, whereas lesions with negative physiology can be safely deferred. It seems that this basic principle holds true regardless of whether FFR or iFR is used for clinical decision-making.

Furthermore, iFR showed excellent diagnostic test characteristics when compared with other invasive and non-invasive measures of stenosis severity and myocardial ischaemia such as the hyperaemic stenosis resistance index, coronary flow reserve (CFR), and positron emission tomography [21].

Conclusion

This study demonstrates that iFR pullback performed under resting conditions predicted the physiological outcome of PCI with a high degree of accuracy. Compared with angiography alone, availability of iFR pullback data significantly decreased the number and length of hemodynamically significant lesions identified for revascularization.

Limitations:

- The selection bias cannot be excluded because the patient enrollment was not totally consecutive and the selection of target vessels was left to the discretion of operators. However, patients were prospectively recruited for a short period of time to minimize the bias.
- Although we assessed iFR gradients on the basis of coronary segments, it also might have been possible to assess them based on coronary lesions. However, since our target vessels consist of not only tandem but also focal and diffuse lesions, the segmental assessment was the only way to assess the reproducibility in our population.
- Potential sources of operator error include differences in the mental coregistration of the angiographic position of the pressure gradient on the iFR pullback curve. In this study, operators were required to observe physiological pullback data and angiographic information at the same time and mentally coregister the 2 pieces of information.
- Furthermore, visual angiographic grading of lesion length is likely to have varied between operators, but this practice remains representative of routine clinical care.

Financial support and sponsorship
Nil

Statement on ethical issues
Research involving people and/or animals is in full compliance with current national and international ethical standards.

Conflict of interest
None declared.

Author contributions
The authors read the ICMJE criteria for authorship and approved the final manuscript.

References
3. Ong AT, van Domburg RT, Aoki J, Sonnenschein K, Lemos PA, Serruys PW. Sirolimus-eluting stents remain superior to bare-metal stents at two years: midterm
results from the Rapamycin-Eluting Stent Evaluated at Rotterdam Cardiology Hospital (RESEARCH) registry. J Am Coll Cardiol 2006; 47:1356–60.
10. Härle T, Bojara W, Meyer S. Comparison of instantaneous wavefree ratio (iFR) and fractional flow reserve (FFR)—first real world experience. Int J Cardiol 2015;199:1–7
Features of the course of cardiovascular diseases against the background of COVID-19


Kabardino-Balkarian State University named after H.M. Berbekov, 360004, Russia, Kabardino-Balkarian Republic, Nalchik, Chernyshevsky St., 173

*Corresponding author: k.zareta.7@mail.ru

Abstract

Based on the analysis of 308 electronic medical records of patients with a confirmed diagnosis of a new coronavirus infection COVID-19, the features of the course of cardiovascular diseases at the regional level were studied. It was found that in patients with cardiovascular diseases, the severity of the course and mortality are higher than in patients without cardiac pathology.

Keywords

COVID-19, Cardiovascular diseases, Features of the course, Treatment

Relevance of the problem

The results of studies by researchers both in Russia and abroad indicate that damage to the cardiovascular system (CVS) is observed in 20-30% of patients with COVID-19 [1, 2, 3]. The combination of CVD and COVID-19 produces additional difficulties in diagnosing, determining priority tactics and choosing therapy. Along with the direct cardiotropic effect of the SARS-CoV-2 virus, pro-arrhythmic effects of some drugs used to treat COVID-19 are also possible. For example, it is well known that antiviral drugs have a potential arrhythmogenic effect due to their ability to prolong the QT interval, especially in patients with an initial increase in the QT interval, which increases the risk of arrhythmic death [4,5].

In addition, an increase in the incidence of cardiovascular events after recovery from COVID-19 may also play a role in the increase in mortality [6,7,8].

Despite numerous studies by international and Russian scientists, the problem of cardiovascular diseases against the background of COVID-19 has not been fully studied. There are no convincing data on the impact of individual factors on survival and long-term life prognosis. In particular, the extent and nature of the MTR burden in this population is not fully understood and remains open.

The situation is complicated by a lack of information, a significant volume of contradictory publications on this issue, and the importance of information for clinical practice. The available information currently consists of an analysis of small observational studies, speculations about possible mechanisms of the pathological impact of the infection on the cardiovascular system, and prospects for treatment and prevention [9, 10].

Further improving the effectiveness of medical care for patients with COVID-19 at the present stage is one of the important tasks. Therefore, the study of regional features of the course of cardiovascular pathology will contribute to improving the quality and effectiveness of medical care for patients with CVD in the context of SARS-CoV-2 infection, both at the hospital stage and in the post-COVID period.

Material and methods

The study is based on the results of a retrospective analysis of electronic medical records of 308 patients with verified diagnosis of COVID-19 (51.9% men, 48.1% women).

From 14.04.2020 till 31.03.2021, 6 541 people were admitted to the hospital of especially dangerous infections No. 2 in Nalchik, among them 477 people died (7.3%). All patients included in the study were divided into 2 groups: the first group (n=182) consisted of patients with CVD, the second the patients (n=126) without CVD.

SARS-CoV-2 infection was determined by polymerase chain reaction (RT-PCR) using the CoV-2 Test
kit. Clinical and laboratory examination was carried out in accordance with medical and economic standards using standard methods by qualified laboratory specialists. In addition, the QT interval was analyzed and monitored, especially in the case of QT-prolonging interval therapy.

Research results
The structure of CVD and COVID in the COVID-19 patients is shown in Figure 1 herein.

Arterial hypertension (AH) was most often detected among COVID-19 patients, which was recorded in 93.4% of the patients, and 66.5% had grade 3 hypertension. On the 2nd place there was recorded chronic heart failure (CHF) (60.9%), and on the 3rd reported were cardiac arrhythmias (40.1%). Ischemic heart disease (CHD) was observed in 21.9% of the patients, post-infarction cardioclesis was recorded in 10.4% of cases.

Our analysis of the severity of COVID-19 revealed the following: moderate course was observed in 112 (36.4%), severe – in 88 (28.5%) and extremely severe – in 108 (35.1%) patients. There were statistically significant differences in the severity of infection depending on the duration of hospitalization (Table 1).

The table given herein shows that mortality was higher in the group of patients admitted to the hospital (n=112) on day 5-7 of illness (59.8%). The second group in terms of mortality was the group of patients admitted on day 8 or later (n=149) (47.6%). A favorable course was observed among patients admitted at an earlier stage of the disease (63.8%). The structure of patients with complications was dominated by patients hospitalized at a later stage of the disease in 84.8-90.6% of the cases (Table 2).

Table 1
Features of the course depending on the terms of hospitalization

<table>
<thead>
<tr>
<th>Sign</th>
<th>1-3 days from the day of the disease (n=47)</th>
<th>5-7 days from the day of the disease (n=112)</th>
<th>8 or more days from the day of the disease (n=149)</th>
<th>P1 (1-3 days and 5-7 days)</th>
<th>P2 (1-3 days and 8 or more days)</th>
<th>P3 (5-7 days and 8 or more days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bed-day</td>
<td>14.7</td>
<td>13.3</td>
<td>13.3,3</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Recovery</td>
<td>5 (10,6%)</td>
<td>9 (8,0%)</td>
<td>9 (6,0%)</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Improvement</td>
<td>30 (63,8%)</td>
<td>36 (32,1%)</td>
<td>68 (45,6%)</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Death</td>
<td>12 (25,5%)</td>
<td>67 (59,8%)</td>
<td>71 (47,6%)</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Complications</td>
<td>36 (76,6%)</td>
<td>95 (84,8%)</td>
<td>135 (90,6%)</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Table 2
Correlation between the severity of COVID-19 and the presence of cardiovascular pathology

<table>
<thead>
<tr>
<th>Indicators of hypertension</th>
<th>AH+CHD</th>
<th>CHD</th>
<th>P1 (AH and AH+CHD)</th>
<th>P2 (HYPERTENSION and CHD)</th>
<th>P3 (AH+CHD and CHD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age group</td>
<td>68.5±1.03</td>
<td>72.1±1.7</td>
<td>70.4±2.2</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>Bed-day</td>
<td>13.7±0.7</td>
<td>12.4±1.7</td>
<td>11.7±2.7</td>
<td>&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>Fever</td>
<td>37.7±0.3</td>
<td>39.4±2.3</td>
<td>37.1±0.2</td>
<td>&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>NPV</td>
<td>24.2±0.7</td>
<td>23.6±0.6</td>
<td>23.3±0.7</td>
<td>&lt;0.01</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>SPO2</td>
<td>88.5±0.5</td>
<td>88.8±1.2</td>
<td>91.8±1.3</td>
<td>&lt;0.01</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>pulse</td>
<td>88.5±1.2</td>
<td>85±2.1</td>
<td>86.2±3.9</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>CRP</td>
<td>55.7±2.3</td>
<td>85.5±21.7</td>
<td>70.3±8.1</td>
<td>&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>Procalcitonin</td>
<td>15 (10.6%)</td>
<td>1 (3.4%)</td>
<td>3 (25%)</td>
<td>&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>LDH</td>
<td>342.2±16.1</td>
<td>358.3±311.2</td>
<td>474±56.8</td>
<td>&lt;0.05</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ferritin</td>
<td>588.2±24.6</td>
<td>664.4±65.3</td>
<td>831±71.4</td>
<td>&lt;0.05</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>IL-6</td>
<td>179.3±12.5</td>
<td>172±211.1</td>
<td>267.0±122</td>
<td>&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>D-dimer</td>
<td>260.7±39.6</td>
<td>385.2±87.7</td>
<td>1182±41.2</td>
<td>&lt;0.05</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>CT scan at admission</td>
<td>43.9±1.7</td>
<td>42.5±3.5</td>
<td>38.5±5.0</td>
<td>&lt;0.05</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Dynamic CT</td>
<td>61.3±2.2</td>
<td>70.0±4.7</td>
<td>68.7±6.7</td>
<td>&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>Complications</td>
<td>131 (92.9%)</td>
<td>28 (96.6%)</td>
<td>12 (100%)</td>
<td>&lt;0.05</td>
<td></td>
</tr>
</tbody>
</table>

Figure 1. Structure of CVD in COVID patients (n=182)
As can be seen from the table, there is a significant correlation between the presence of CVD and the severity of the disease in patients with COVID-19. Moreover, in patients of older age categories, these connections are more pronounced. Particular attention should be drawn to a more pronounced increase in inflammatory markers in patients with AH and AH+CHD (Table 3).

**Table 3**
Comparative analysis of inflammatory markers and blood coagulation parameters in the study groups

<table>
<thead>
<tr>
<th>Indicators</th>
<th>Group 1</th>
<th>Group 2</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP</td>
<td>61.5±3.9</td>
<td>52.2±3.2</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Procalcitonin</td>
<td>19 (10.4%)</td>
<td>5 (3.9%)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>LDH</td>
<td>333.5±14.1</td>
<td>294.5±17.4</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Ferritin</td>
<td>616.3±24.7</td>
<td>425.6±23.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IL-6</td>
<td>183.9±11.9</td>
<td>82.8±10.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PTI</td>
<td>94.5±4.8</td>
<td>92.7±1.1</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>INR</td>
<td>1.2±0.03</td>
<td>3.5±1.4</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>APTT</td>
<td>27.4±0.7</td>
<td>23.9±0.6</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>D-dimer</td>
<td>341.3±45.8</td>
<td>80.8±26.1</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>519.9±24.3</td>
<td>526.5±19.3</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

It was found that in patients with CVD, the level of inflammatory markers and the tendency to hypercoagulability were significantly higher.

The results of the comparative analysis of the frequency and structure of complications in the study groups are presented in Table 4 herein.

**Table 4**
Structure of complications in patients with COVID-19

<table>
<thead>
<tr>
<th>Complication</th>
<th>Group 1 n=182</th>
<th>Group 2 n=126</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhythm disorders</td>
<td>49 (26.9%)</td>
<td>58 (46.0%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Conduction</td>
<td>59 (32.4%)</td>
<td>16 (12.7%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>RF 1</td>
<td>15 (8.2%)</td>
<td>31 (24.6% per cent)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>RF 2</td>
<td>33 (18.1%)</td>
<td>35 (27.8% per cent)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>RF 3</td>
<td>89 (48.9%)</td>
<td>21 (16.7%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>NHTeal</td>
<td>66 (36.3%)</td>
<td>7 (5.6%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SD</td>
<td>Card Holder 19 (10.4%)</td>
<td>8 (6.3%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TO THEM</td>
<td>13 (7.1%)</td>
<td>2 (1.6%)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>ONMK</td>
<td>23 (12.6%)</td>
<td>3 (2.4%)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Acute thrombosis of the lower extremities</td>
<td>2 (1.1%)</td>
<td>2 (1.6%)</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

The table shows that the patients with CVD were significantly more likely to have more severe complications (stage 3 RF (48.9%), PE (36.3%), ARDS (20.9%), DM (10.4%)). compared to those in group 2 of the study. Conduction disturbances and RF of the 3rd degree were observed 3 times more often in patients of the 1st group than in the 2nd, PE more than 5 times, MI 4.5 times, sepsis 8 times, hydropericardia 7 times.

Given that the female sex prevailed in the structure of patients, the gender features of the COVID-19 course were studied. The results are shown in Table 5.

**Table 5**
Gender features of the COVID-19 course

<table>
<thead>
<tr>
<th>Indicators</th>
<th>Men (n=143)</th>
<th>Women (n=165)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>63.6±1.9</td>
<td>64.2±1.2</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Bed-day</td>
<td>13.6±0.6</td>
<td>13.5±0.7</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Temperature</td>
<td>38.2±0.5</td>
<td>38.2±0.5</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>BPD</td>
<td>23.5±0.6</td>
<td>23.0±0.4</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>GARDEN</td>
<td>131.2±23.1</td>
<td>133.9±23.1</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>dad</td>
<td>79.9±11.9</td>
<td>81.3±7.2</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>SPO2</td>
<td>89.3±0.5</td>
<td>89.3±0.4</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>pulse</td>
<td>87.9±1.1</td>
<td>89.2±1.1</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>SBP</td>
<td>133.9±16.8</td>
<td>135±14.6</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Ferritin</td>
<td>585.9±9.5</td>
<td>497±21.8</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>IL-6</td>
<td>162.9±14.1</td>
<td>124.9±10.5</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>D-dimer</td>
<td>360.3±56.8</td>
<td>125.9±23.7</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>508.1±16.6</td>
<td>534.9±6.8</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>CT scan at admission</td>
<td>39.1±1.5</td>
<td>41.9±1.5</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>NSP</td>
<td>59.3±2.3</td>
<td>48.7±2.3</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Complications</td>
<td>130 (90.9%)</td>
<td>135 (81.9%)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Improvement</td>
<td>52 (36.4%)</td>
<td>83 (50.3%)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Recovery</td>
<td>7 (4.9%)</td>
<td>16 (9.7%)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Fatality rate</td>
<td>84 (58.7%)</td>
<td>66 (40%)</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>
There were no significant differences in age, body temperature, saturation, BMI, and bed days among men and women. However, the indicators of inflammatory markers were higher in men. Significant deterioration in lung CT parameters was also observed among males. Although the number of complications was dominated by women, the outcomes were more severe among men: improvement was observed in 36.4% of the cases among men, and in 50.3% among women. The mortality rate among men was 58.7%, among women-40%.

Our analysis to study hospital mortality from COVID-19 included 150 deaths (66 (44%) women, mean age 50 ± 19.5 years, 84 men (56%), mean age 50 ± 19.4). The age structure of the deceased was dominated by people aged 60-69 years with predominance of males, and in the age group of 70-79 years women. Among people 80 years and older, there were 2 times more men than women. The structure of causes of death is shown in Table 6 herein.

Table 6

<table>
<thead>
<tr>
<th>Complications</th>
<th>Total patients</th>
<th>% (n=150)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pulmonary embolism</td>
<td>66</td>
<td>44</td>
</tr>
<tr>
<td>Acute respiratory distress syndrome</td>
<td>33</td>
<td>22</td>
</tr>
<tr>
<td>Myocarditis</td>
<td>8</td>
<td>5.3</td>
</tr>
<tr>
<td>Hydropericard</td>
<td>24</td>
<td>16</td>
</tr>
<tr>
<td>Acute cerebrovascular accident</td>
<td>10</td>
<td>6.6</td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>12</td>
<td>8</td>
</tr>
<tr>
<td>Acute kidney injury</td>
<td>31</td>
<td>20.6</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>15</td>
<td>10</td>
</tr>
<tr>
<td>Sepsis</td>
<td>16</td>
<td>10.6</td>
</tr>
<tr>
<td>Pneumothorax</td>
<td>8</td>
<td>5.3</td>
</tr>
<tr>
<td>Pneumomediastinum</td>
<td>1</td>
<td>0.6</td>
</tr>
</tbody>
</table>

As can be seen from the Table, the structure of causes of death was dominated by pulmonary embolism (44%), acute respiratory distress syndrome (22%), acute kidney injury-20.6%, hydropericardia-16%, sepsis-10.6%, pneumothorax-5.3%. These indicators differ from the results of studies by other authors that again confirms the importance of studying the regional features of the course of COVID-19.

Conclusions

1. Study of the prevalence of cardiovascular diseases among the studied population of patients with COVID-19 showed: arterial hypertension was recorded in 93.4% of the patients, chronic heart failure in 60.9% of the cases, cardiac arrhythmias-in 40.1% of the cases, coronary heart disease in 21.9% of the patients, post-infarction cardiosclerosis in 10.4% of the cases.

2. A clear dependence of the severity of the course and outcome of the disease on the time of hospitalization was revealed: a favorable course was noted among patients admitted at an earlier stage of the disease. Among patients with severe complications, the patients who were hospitalized at a later stage of the disease prevailed.

3. In patients with CVD, the level of inflammatory markers and the tendency to hypercoagulability were significantly higher.

4. In patients with CVD, an unfavorable course of COVID-19 was observed with a large number of complications: cardiac arrhythmias and respiratory failure of 3 degrees were observed 3 times more often than in patients without CVD, PE-more than 5 times, MI-4.5 times, sepsis-8 times, hydropericardia-7 times.

5. Gender differences in the course and outcomes of COVID-19 were revealed: the severity of the course and mortality rates were higher among males.

6. CVD and its complications, such as hypertension, CHD, CHF, cardiac arrhythmias, PE, and brain strokes, made the greatest contribution to the structure of causes of hospital mortality.

Practical recommendations

1. Data on the prevalence of CVD and its complications among patients with COVID-19 were presented to the Practical Healthcare Department of the Kabardino-Balkar Republic. The analysis performed was carried out in one hospital serving most of the urban population and districts of the republic, so the results obtained cannot be extrapolated to patients with COVID-19 throughout the republic.

2. The results obtained will allow optimizing the tactics of providing medical care to patients with CVD during the COVID-19 pandemic and can be used in developing prevention programs and improving the rehabilitation of patients in the post-COVID-19 period.

3. The high frequency of thromboembolic complications may be related to the low adherence of patients to anticoagulants in the presence of atrial
fibrillation and other indicated cases in the pre-
COVID-19 period, and therefore it is necessary to
increase the effectiveness of dispensary monitoring
of patients with atrial fibrillation (open offices of
patients with AF in polyclinics).

4. The results obtained, which indicate the presence
of regional features of the prevalence and course
of CVD in patients with COVID-19, should be
taken into account when planning rehabilitation
measures both at the hospital stage and at the post-
covid rehabilitation stage.

5. Further improving the effectiveness of medical care
for patients with COVID-19 at the present stage is
one of the most important tasks. Therefore, the
study of regional features of the course and treat-
ment of cardiovascular pathology will help reduce
the mortality of this category of patients.

Statement on ethical issues
Research involving people and/or animals is in full
compliance with current national and international
ethical standards.

Conflict of interest
None declared.

Author contributions
The authors read the ICMJE criteria for authorship
and approved the final manuscript.

References
1. Bazdyrev ED. Coronavirus infection – an actu-
al problem of the XXI century. Complex problems
of cardiovascular diseases. 2020; 9(2):6-16. DOI:
10.17802/2306-1278-2020-9-2-6-16.
2. Barbarash OL, Karetnikova VN, Kashtalap VV, et
al. New coronavirus disease (covid-19) and cardiovas-
cular diseases. Complex problems of cardiovascular
Et al. Recommendations of the Eurasian Arrhyth-
moilogical Association (EURA) for the diagnosis and
treatment of patients with cardiac arrhythmia and
conduction disorders during the COVID-19 pandem-
ic. Cardiology. 2020;60(5):4-8.
4. Grinevich VB, Gubonina IV, Doschtsin VL, et
al. Features of management of comorbid patients
during the new coronavirus infection (covid-19)
pandemic Cardiovascular therapy and prevention.
5. Polonskaya YV, Kashtanova EV, Stakhneva EM, et
al. Covid-19 and cardiovascular diseases. Atheroscle-
rosis. 2020;16(2):73-9.
6. Chazova IE, Mironova OYu. COVID-19 and car-
7. Shlyakhto EV, Konradi AO, Villevalde SV. Guide-
lines for the diagnosis and treatment of circulatory sys-
tem diseases in the context of the COVID-19 pandem-
International Journal of Heart and Vascular Diseases.
vascular disease and myocardial injury with outcomes
of patients hospitalized with 2019-coronavirus disease
(COVID-19). JAMA Cardiol. Published online March
10. Madjid M, Safavi-Naeini P, Solomon SD, Vardeny
O. Potential Effects of Coronaviruses on the Cardio-
vascular System: A Review. JAMA Cardiol. Published
online March 27, 2020. DOI: 10.1001/ jamacar-
dio.2020.1286.
The state of the cardiovascular system in women during pregnancy

Vakha A. Anzorov*, Svetlana V. Moryakina

Chechen State University named after A.A. Kadyrov, 364024, Russia, Grozny, Sheripova str., 32

*Corresponding author: vaha-anzorov@mail.ru

Abstract
The work is devoted to the study of the impact of pregnancy on the state of the cardiovascular system in women. The process of pregnancy is accompanied by significant changes in the rhythm of the heart performance and blood pressure, and the duration of the ECG waves and segments are not significantly fluctuated. The heart rhythm, gradually increasing, reaches its maximum value of 87.3 beats per minute (P < 0.001) in the last trimester, in women of the reference group 69.8. The minimum level of systolic blood pressure is 105.6 mm Hg, diastolic 69.5 mm Hg, detected in the second trimester of pregnancy, and the maximum - 146.4 and 88.0 mm Hg, respectively in the third one. The time of the P wave during pregnancy tends to decrease. The minimum time 0.151 s and maximum 0.162 in the reference is used for excitation from the atria to the ventricles in the third trimester. The coverage time of the ventricular excitation during pregnancy is reduced. Thus, the excitation in the third trimester occurred in 0.077 s, and 0.083 s in the reference group. In the reference group of women, the duration of QT was 0.346 s, and 0.331 s by the end of pregnancy.

Keywords
Pregnancy, Heart rate, Blood pressure, ECG indicators

Imprint

The social and economic problems which remain unsolved for the population is the main cause of high mortality and low birth rates.

Due to the demographic problems which Russia has been facing in the last few decades, the birth of every healthy baby is a big event that requires appropriate attention.

The state authorities recognize this fact and provide material support to every young family in which children are born and raised.

The decision to have a first pregnancy, due to the uncertainty of its consequences, poses a huge risk for a woman.

During the period of her life, a woman does not have to face such fears and anxieties.

In order to successfully survive this time, every woman should be aware of possible changes, both in her own body and the fetus.

Even in the old days in Russia, it was believed that in order to give birth to a healthy child, the expectant mother during the conception period should avoid looking at freaks and cripples.

Being in the womb, the future person feels the state of the mother, her experiences, sensations, thoughts and draws conclusions about the world: good; bad; accept me or not; poses a danger to me or not, and so on. The level of mother’s love shown during pregnancy determines the state of the psyche of the fetus, its memory and its qualities as individuals that persist throughout life [3].

During this period, the following is strictly prohibited: fatigue; high physical activity; night duty; performance of work with body vibration and the influence of substances with a sharp odor on the body. The duration of sleep should be not less than 8 hours, and it should be preceded by a walk. A pregnant woman should isolate herself from infection with infectious diseases. She should wear comfortable and loose clothing, avoid constricting belts and tight bras.

To support the abdomen in the second half of pregnancy, it is necessary to wear a bandage. Shoes should not be high heels.

To preserve the health of a woman and ensure the proper development of the fetus, proper nutrition is important. The use of a diet is not mandatory for women in the first half of pregnancy, however, the food should be tasty and varied.

During pregnancy, a woman should eat at least six times a day. Since proteins are the plastic (building) material of the fetus, they play a special role in the nutrition of a pregnant woman.

High nutritional value of animal proteins should be noted that enter the mother’s body with foods such

The social and economic problems which remain unsolved for the population is the main cause of high mortality and low birth rates.
as eggs, meat, fish and dairy products. The need of a pregnant woman is also high in products containing plant proteins. These are soy and nut proteins.

In the diet, preference should be given to fruits, berries and vegetables. Fats should be used in the form of butter, sour cream and vegetable oils. During pregnancy, the need for vitamins increases, and therefore foods rich in vitamins should predominate in the diet.

Advances in the development of physiology put forward in the relationship between the mother and the fetus, the need to reassess the data on the functional activity of body systems [11].

This need is due to the fact that the mother and the fetus are two independent organisms, which are united by the satisfaction of the needs of the fetus, which ensure the normal development of the embryo.

Deviations in the embryonic development of the fetus can be caused both by disturbances in the genetic apparatus of the fetus, and by the influence of some environmental factors. This means that the optimal state of environmental conditions alone is not enough to ensure the normal development of the fetus.

Also, for this, it is necessary to maintain the constancy of the internal environment in the mother-fetus system, which ensures the development of the fetus according to the genetic plan.

Identification of changes occurring in the body of a pregnant woman will allow us, through preventive measures, to avoid the development of possible pathologies [14]. A woman’s illness during pregnancy poses a threat not only to the body of the expectant mother, but also to the normal development of the fetus.

Therefore, the determination of indicators of the cardiovascular system, as one of the most important in the body, during pregnancy is important and relevant.

In order to identify changes in the cardiovascular system of women during pregnancy, we conducted research in the maternity department of the Urus-Martan district hospital.

Materials and methods
The object of our research were 50 clinically healthy women, whose age ranged from 23 to 25 years. Among them, two groups were formed, each group consisted of 25 women. Women in the reference group did not have a pregnancy, while the experimental ones were pregnant, and its duration was the same.

The conditions for the inclusion of women in the experimental group were their consent and the absence of contraindications for the course of pregnancy. Blood samples were taken at the end of each trimester of pregnancy, in the morning before taking water and food.

Heart rate and arterial blood pressure values were determined using expert digital automatic tonometer OMRON M3.

Electrocardiogram parameters were recorded using an Alton-03 electrocardiograph.

The “Biostatistics” software was used for statistical processing of the experimental data.

Results and Discussion
The dynamics of the cardiovascular system indicators in women during pregnancy is shown in Table 1 and in Figures 1 and 2 herein.

<table>
<thead>
<tr>
<th>Indicators</th>
<th>Reference</th>
<th>Trimester of pregnancy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>HR, beats per minute</td>
<td>69.8±1.24</td>
<td>78.6±1.18***</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>117.0±2.03</td>
<td>116.2±1.67</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>78.2±1.41</td>
<td>77.7±1.52</td>
</tr>
<tr>
<td>P, s</td>
<td>0.076±0.0018</td>
<td>0.074±0.0015</td>
</tr>
<tr>
<td>PQ, s</td>
<td>0.162±0.0040</td>
<td>0.158±0.0043</td>
</tr>
<tr>
<td>QRS, s</td>
<td>0.083±0.0014</td>
<td>0.081±0.0025</td>
</tr>
<tr>
<td>QT, s</td>
<td>0.346±0.0033</td>
<td>0.340±0.0044</td>
</tr>
</tbody>
</table>

*** – P < 0,01; **** – P < 0,001
Figure 1. Cardiovascular system in women during pregnancy

Figure 2. Electrocardiogram parameters in women during pregnancy

The table and figures herein show that with the course of pregnancy, the heart rate increases significantly, blood pressure decreases until the second trimester, then increases, ECG parameters do not undergo significant changes.

Thus, the heart rate increased in the first trimester of pregnancy by 8.8 beats per minute \( P < 0.001 \), 11.7 \( (P < 0.001) \) in the second and 17.5 \( (P < 0.001) \) in the third as compared to the reference.

Arterial blood pressure decreased in the first and second trimesters of pregnancy, and increased in the third.

The drop in systolic pressure in the 1st and 2nd trimesters was 0.8 mm Hg and 11.4 \( (P < 0.001) \), and in the diastolic one 0.5 and 8.7 \( (P < 0.01) \), as compared to non-pregnant women.

In the third trimester, the level of systolic pressure exceeds the initial one by 29.4 mm Hg \( (P < 0.001) \) and the level of diastolic pressure by 9.8 \( (P < 0.01) \).

Apparenty, a significant increase in heart rate and fluctuations in blood pressure during pregnancy is a consequence of stimulation of the cardiac activity, as well as a decrease in peripheral blood pressure due to the uteroplacental circulation [12].

Our data are also confirmed by other authors. So, in the second trimester of pregnancy, due to a decrease in peripheral vascular resistance owing to an additional uteroplacental circulation, there is a decrease in blood pressure by 8-15 mm Hg, and in the third an increase therein, but no more than 10-15% [5].

During pregnancy, there is an increase in cardiac output by 30-40%, and at the same time, the activity of the left ventricle is enhanced [7].

Joanna S. [10] reports on the basis of her research that in the first trimester of pregnancy there is an increase in the heart rate by 5-7 beats per minute, and by the end of the third by 15-20.

According to A.A. Kikshun [4], with the development of pregnancy, the stroke volume of the heart increases by 30% and the heart rate by 15-20 beats per minute. From the beginning of pregnancy, the pulse value increases by 10-15 beats per minute and remains at this level until childbirth [1, 13]. Such factors of the pregnancy process as an increase in the amount of blood in the vascular system and the appearance of additional blood circulation that feeds the fetus enhance the load on the heart and blood vessels [6].

Simkin P, et al [9] found that during pregnancy there was an increase in the amount of circulating blood by 40-55%. At the beginning of pregnancy, the increase in circulating blood volume was 30-45%.

In the absence of pathologies, arterial blood pressure (BP) does not undergo to significant changes during pregnancy.

From the beginning of pregnancy, there is an increase in cardiac output, reaching its maximum value of 6-7 l/min by the middle of the third trimester [1].

The duration of P, PQ, QRS and QT intervals decreases slightly with the course of pregnancy. Thus, the shortening of the P wave in the third trimester was 0.006 seconds.

The time of excitation from the atria to the ventricles decreased by 0.011 s at the end of pregnancy.

The difference between the extreme values of the duration of the QRS wave was 0.006 seconds.

The ventricular systole in the third trimester took 0.015 s less time than that in the reference women.
In the normal course of pregnancy, the electrocardiogram parameters do not undergo to any significant changes [2].

According to Savelieva G.M., Sukhikh G.T., Serov V.N. and Radzinsky V.E. [8], pregnancy is accompanied by significant changes in the performance of the organs and systems in the woman’s body.

They are adaptive in nature and are aimed both at meeting the needs of the growing fetus and at creating the necessary conditions for a favorable course of the birth process.

Thus, pregnancy leads to a significant increase in the heart rate, significant fluctuations in blood pressure in both directions and an unreliable reduction in the time of electrocardiogram indicators.

Conclusions

1. During pregnancy, women experience significant changes in heart rate and blood pressure.
2. Heart rate increases in the first trimester by 12.6%, in the second and third ones by 16.8 and 25.1%, respectively.
3. Systolic blood pressure drops in the second trimester by 9.7%, and the diastolic one by 11.2%.
4. An increase in systolic blood pressure by the end of pregnancy was 25.1%, and that in the diastolic pressure reached 12.5%.
5. Duration of the waves and segments of the electrocardiogram during pregnancy is slightly reduced.

Statement on ethical issues

Research involving people and/or animals is in full compliance with current national and international ethical standards.

Conflict of interest

None declared.

Author contributions

The authors read the ICMJE criteria for authorship and approved the final manuscript.

References

Prevalence of anxiety and depression disorders among medical students one year after the start of the COVID-19 pandemic

Aksana M. Kardangusheva*, Diana A. Dzakhmysheva, Milana A. Kardanova, Amina Y. Chanaeva, Marina A. Makoeva, Ilona S. Khagabanova

Kabardino-Balkarian State University named after H.M. Berbekov, 360004, Russia, Kabardino-Balkarian Republic, Nalchik, Chernyshevsky St., 173

*Corresponding author:
kardangush@mail.ru
+7(964)0311050

Abstract

Aims. The aim is to study the incidence rate of anxiety and depressive disorders and their relationship with the everyday activity at the H.M. Berbekov Kabardino-Balkarian State University (KBSU) medical faculty students a year after the start of the Covid-19 pandemic. As a part of a cross-sectional observational study, a survey of 425 students at the Faculty of Medicine was conducted. The average age of the surveyed students was 20.9±2.1 years. The Spielberger-Khanin questionnaire, the HADS anxiety and depression scale, as well as a questionnaire specially developed by the authors to clarify some indicators of students' activity were used.

Results. The prevalence of high levels of situational anxiety (SA) was 1.4%, personal anxiety (PA) 30.8%, anxiety 29%, depression 21.6%. High levels of SA were statistically significantly more common among students with higher education (p = 0.0417), living in rented apartments (p = 0.0136), countryside residents (p = 0.0005), with debts (p = 0.0122); high levels of PA were identified among students who assessed their living conditions as satisfactory (p=0.0001). Clinically pronounced depression was statistically significantly more common among students with higher education (p=0.0258), who assess their living conditions as good (p=0.028), have debts (p=0.0001), employed (p=0.0001) and practiced low physical activity (p=0.0001). Associative relationships were detected between the levels of SA, PA, anxiety, depression and students' activity parameters included in the study.

Conclusion. The results obtained can be used to improve the academic process and preserve the mental health of the KBSU medical faculty students.

Keywords
Students, Situational anxiety, Personal anxiety, Depression, Anxiety, Pandemic, New coronavirus infection Covid-19

Imprint

On March 11, 2020, the World Health Organization declared Covid-19 global pandemic. Taking into account the Covid-19 spread, unprecedented social measures have been taken in most countries to control the disease. Educational institutions have had to transit from traditional classroom learning to distance learning or hybrid learning during times of upsurge in Covid-19 and contact learning during times of decline in Covid-19. Such an organization of the educational process made it possible to ensure the continuity of education. However, many questions arose both on the pedagogical aspects of vocational education in the new conditions, and on the new risks to the health of students. Currently, the results of scientific studies concerning the adaptation of students to online learning and the impact of Covid-19 on the mental health of students have been published [1-8]. In our opinion, this problem is especially relevant for students enrolled in medical specialty programs due to the impossibility of their practical training in distance learning [1]. Despite the fact that some studies point to the negative impact of the pandemic and restrictive measures on the mental health of students, the impact of the pandemic on university education and on the health of medical students remains insufficiently studied. In our study, we focused more on studying the prevalence of signs of personal and situational anxiety, anxiety and depression in medical students during the Covid-19 pandemic.

Aims: to study the incidence rate of anxiety and depressive disorders and their relationship with everyday activities at the H.M. Berbekov Kabardino-Balkarian State University (KBSU) medical faculty students a year after the start of the Covid-19 pandemic.
Materials and methods

The study included the following stages: 1) theoretical: analysis of scientific literature and formation of a methodological basis for the study; 2) development of a special questionnaire; 3) conducting research, 3) mathematical statistical analysis.

The study was conducted at the Faculty of Medicine at the KBSU in 2021. A cross-sectional observational study included 425 students (144 males and 281 females) of the specialty “General Medicine” at the Faculty of Medicine, who underwent practical training at clinical bases and training in a contact form and agreed to participate in research. The average age of the surveyed students was 20.9±2.1 years. Socio-demographic characteristics of the surveyed population are presented in Table 1.

The majority of students were not married (93.2%). Only 6.8% of students, among which 24.1% of the males and 75.9% of the females were married (p<0.0001). The main source of income for students (91.1% of females and 55.6% of males, p<0.0001) was the parents’ fund. At the same time, 6% of females and 34% of males (p<0.0001) indicated their earnings as the main source of income, and 2.8% of the females and 10.4% of the males (p<0.01) other sources of income (scholarship, other relatives’ fund). The leading activity of the students is study. At the same time, in our sample, 8.2% of the females and 23.6% of the males (p<0.0001) combined study with their employment. Moreover, 43.5% of employed females and 38.2% of employed males indicated that they have night shifts at work.

A specially designed questionnaire contained questions to assess marital status, education, employment information, physical activity level, self-assessment of health, academic performance, academic debt, preparation time for classes, class duration, sleep duration, smoking and alcohol consumption. To determine the level of situational anxiety (SA) and personal anxiety (PA), testing was carried out according to the Spielberger-Khanin method [9]. When assessing levels of anxiety, a score of less than 30 was assessed as low anxiety; 31 to 45 moderate anxiety and 46 or more as high anxiety [9]. Anxiety and depression levels were measured using the HADS Anxiety and Depression Scale developed by A.S. Zigmond and R.P. Snait in

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Socio-demographic characteristics of the surveyed students</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indicators</td>
<td>Females</td>
</tr>
<tr>
<td>Marital status</td>
<td></td>
</tr>
<tr>
<td>Married</td>
<td>22</td>
</tr>
<tr>
<td>Not married</td>
<td>259</td>
</tr>
<tr>
<td>Education</td>
<td></td>
</tr>
<tr>
<td>Higher</td>
<td>24</td>
</tr>
<tr>
<td>Vocational secondary</td>
<td>6</td>
</tr>
<tr>
<td>Secondary</td>
<td>251</td>
</tr>
<tr>
<td>Place of residence</td>
<td></td>
</tr>
<tr>
<td>Urban</td>
<td>206</td>
</tr>
<tr>
<td>Rural</td>
<td>75</td>
</tr>
<tr>
<td>Type of residence</td>
<td></td>
</tr>
<tr>
<td>Dormitory</td>
<td>11</td>
</tr>
<tr>
<td>Rental apartment</td>
<td>60</td>
</tr>
<tr>
<td>Own apartment</td>
<td>38</td>
</tr>
<tr>
<td>With parents</td>
<td>172</td>
</tr>
<tr>
<td>Housing conditions (according to respondents)</td>
<td></td>
</tr>
<tr>
<td>Excellent</td>
<td>188</td>
</tr>
<tr>
<td>Good</td>
<td>78</td>
</tr>
<tr>
<td>Satisfactory</td>
<td>15</td>
</tr>
<tr>
<td>Source of income</td>
<td></td>
</tr>
<tr>
<td>Parents funds</td>
<td>256</td>
</tr>
<tr>
<td>Own income</td>
<td>17</td>
</tr>
<tr>
<td>Other source</td>
<td>8</td>
</tr>
<tr>
<td>Employment status</td>
<td></td>
</tr>
<tr>
<td>Employed</td>
<td>23</td>
</tr>
<tr>
<td>Having night shifts (from the number of employees)</td>
<td>10</td>
</tr>
</tbody>
</table>

Note: * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001 - statistical significance of differences between males and females
When assessing the levels of anxiety and depression, a score of less than 8 is regarded as “the absence of significantly expressed symptoms of anxiety and depression”, 8-11 as “subclinically expressed anxiety/depression”, 12 and more “clinically expressed anxiety/depression” [10]. Individuals who smoked at least one cigarette per day were considered smokers. The severity of alcohol consumption was determined by the consumption of pure ethanol in grams per week [11]. Physical activity was assessed according to the Global Questionnaire on Physical Activity recommended by the World Health Organization. The group of people with low physical activity (LPA) included students whose moderate-intensity physical activity was less than 150 minutes per week, or high-intensity physical activity less than 75 minutes per week [12].

Statistical analysis of the data was carried out using STATISTICA 6.0 software (StatSoft Inc, USA). The results are presented as the mean and its standard deviation for continuous variables and as a percentage for categorical variables. Hypotheses about a possible relationship between individual quantitative variables were refined by the method of paired correlation analysis. The results of the correlation analysis were interpreted on the basis of the Spearman criterion, while determining the rank correlation coefficient $r$. The values $p<0.05$ were taken as the critical level of significance when testing statistical hypotheses.

Results and Discussion

The levels of SA and PA of the surveyed students are presented in Table 2.

The average SA values of the surveyed students corresponded to low levels, and the average PA values corresponded to the average levels. The prevalence of high levels of PA and moderate levels of SA among females was higher than among males. Low levels of PA were more common among males than among females. Noteworthy is the more favorable profile of SA than PA.

When comparing our data with the results of a study conducted earlier at the Faculty of Medicine [13], a high prevalence of high levels of SA was established both among males and in a group of females in a study conducted earlier, when students studied remotely. However, differences in the prevalence of high levels of PA in studies were insignificant [13]. These differences can be explained by a more pronounced negative effect of external factors in the study of Miziev I.A. et al. (2021), which include distance learning, social restrictions, and a short period of time since the start of the pandemic. It is well known that SA is an adaptive mechanism inherent in all people and occurs in a stressful situation, and PA is a constitutional trait of a person associated with a low threshold of excitability of the nervous system.

Discussing the results obtained by us, it is necessary to note the external factors influencing the level of anxiety. Attention to situationally determined factors is due to the fact that they are controlled. Control and modulation of environmental factors make it possible to bring them as close as possible to the needs of an individual in order to create comfort, increase adaptability, and reduce anxiety. The most favorable profile of anxiety among the students we surveyed was apparently due to the fact that we conducted the study a year after the onset of the pandemic, during the period of students’ internships and their training in a contact format. Following precautions were kept to avoid increasing incidence: social distancing, wearing of masks, etc. However, students may perceive the reduction in restrictive measures as a return to normal life, and having received more information about the pandemic throughout the year, they may also feel more confident.

We studied the prevalence of SA and PA depending on marital status, living conditions, place of residence, etc.

| Table 2 |
|---|---|---|---|---|---|---|
| Levels | Situational anxiety | | Personal anxiety | | |
| | Males | Females | Total | Males | Females | Total |
| Average values, points (M±δ) | 22.4 ± 8.0 | 24.2 ± 8.7 | 23.3 ± 8.4 | 36.6 ± 9.3 | 42.4 ± 9.9 | 39.5 ± 9.6 |
| Low (%) | 82.6 | 77.2 | 79.1 | 30.6 | 9.6**** | 16.7 |
| Moderate (%) | 17.4 | 26* | 19.5 | 53.5 | 52 | 52.5 |
| High (%) | 0 | 2.1 | 1.4 | 15.9 | 38.4**** | 30.8 |

Note: * ($p<0.05$), **** ($p<0.0001$) statistical significance of differences between males and females.
source of income, academic performance, work status, sleep duration, smoking, alcohol consumption, NFA (see Table 3 herein).

In the surveyed population, the prevalence of high levels of SA is statistically significantly higher among people with higher education compared to those with secondary education (p = 0.0417); among students living in rented apartments compared with those living with their parents (p=0.0136); among rural residents, compared with urban residents (p=0.0005), as well as among students with debts, compared with those without them (p=0.0122). High levels of PA were statistically significantly more often recorded in the group of students who rated their living conditions as

Table 3

<table>
<thead>
<tr>
<th>Indicators</th>
<th>SA levels (%)</th>
<th>PA levels (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>low</td>
<td>high</td>
</tr>
<tr>
<td>Family status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>married</td>
<td>82.8</td>
<td>13.8</td>
</tr>
<tr>
<td>not married</td>
<td>78.8</td>
<td>19.9</td>
</tr>
<tr>
<td>Education</td>
<td></td>
<td></td>
</tr>
<tr>
<td>higher</td>
<td>69.3</td>
<td>25.6</td>
</tr>
<tr>
<td>vocational secondary</td>
<td>75</td>
<td>25</td>
</tr>
<tr>
<td>secondary</td>
<td>80.2</td>
<td>18.8</td>
</tr>
<tr>
<td>Type of residence</td>
<td></td>
<td></td>
</tr>
<tr>
<td>dormitory</td>
<td>64</td>
<td>36</td>
</tr>
<tr>
<td>own apartment</td>
<td>79</td>
<td>21</td>
</tr>
<tr>
<td>with parents</td>
<td>81.5</td>
<td>17.7</td>
</tr>
<tr>
<td>rental apartment</td>
<td>76.2</td>
<td>19.0</td>
</tr>
<tr>
<td>Housing conditions</td>
<td></td>
<td></td>
</tr>
<tr>
<td>excellent</td>
<td>80.8</td>
<td>17.7</td>
</tr>
<tr>
<td>good</td>
<td>77.7</td>
<td>22.3</td>
</tr>
<tr>
<td>satisfactory</td>
<td>68.2</td>
<td>27.3</td>
</tr>
<tr>
<td>Place of residence</td>
<td></td>
<td></td>
</tr>
<tr>
<td>countryside</td>
<td>78.1</td>
<td>17.1</td>
</tr>
<tr>
<td>city</td>
<td>79.4</td>
<td>20.3</td>
</tr>
<tr>
<td>Source of income</td>
<td></td>
<td></td>
</tr>
<tr>
<td>parents' funds</td>
<td>78.3</td>
<td>20.2</td>
</tr>
<tr>
<td>Own income</td>
<td>78.8</td>
<td>19.7</td>
</tr>
<tr>
<td>Other income</td>
<td>91.3</td>
<td>8.7</td>
</tr>
<tr>
<td>Academic performance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>excellent</td>
<td>93.2</td>
<td>6.2</td>
</tr>
<tr>
<td>good</td>
<td>79.4</td>
<td>18.8</td>
</tr>
<tr>
<td>satisfactory</td>
<td>72.1</td>
<td>27.9</td>
</tr>
<tr>
<td>Academic debt</td>
<td></td>
<td></td>
</tr>
<tr>
<td>yes</td>
<td>75.3</td>
<td>21.4</td>
</tr>
<tr>
<td>no</td>
<td>80.1</td>
<td>19.3</td>
</tr>
<tr>
<td>Employment status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Employed</td>
<td>80.7</td>
<td>19.3</td>
</tr>
<tr>
<td>Unemployed</td>
<td>78.8</td>
<td>19.6</td>
</tr>
<tr>
<td>Night shifts</td>
<td></td>
<td></td>
</tr>
<tr>
<td>yes</td>
<td>68</td>
<td>32</td>
</tr>
<tr>
<td>no</td>
<td>85.3</td>
<td>24.7</td>
</tr>
<tr>
<td>Sleep duration</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Less than 8 h</td>
<td>75.9</td>
<td>22.2</td>
</tr>
<tr>
<td>8 h and more</td>
<td>84.3</td>
<td>15.1</td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
</tr>
<tr>
<td>yes</td>
<td>76</td>
<td>24</td>
</tr>
<tr>
<td>no</td>
<td>79.3</td>
<td>19.2</td>
</tr>
<tr>
<td>Alcohol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>yes</td>
<td>77.1</td>
<td>21.3</td>
</tr>
<tr>
<td>no</td>
<td>79.4</td>
<td>19.2</td>
</tr>
<tr>
<td>Low physical activity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>yes</td>
<td>73.4</td>
<td>23.4</td>
</tr>
<tr>
<td>no</td>
<td>80.1</td>
<td>18.8</td>
</tr>
</tbody>
</table>

Notes: differences are statistically significant between students, * with higher and secondary education (p = 0.0417); ** living in rented apartments and with parents (p=0.0136); ***living in the city and the countryside (p = 0.0005); ****who have and do not have debts (p = 0.0122); *****who rate their living conditions as satisfactory or excellent (p = 0.0001).
satisfactory compared to peers who rated their living conditions as excellent \( (p=0.0001) \).

Thus, among the surveyed students there is a high prevalence of anxiety. Moreover, the most unfavorable situation is in the group of females compared to males. High levels of SA were more common among students with higher education, living in rented apartments, in rural areas and having debts, and high levels of PA were more common among students who rated their living conditions as satisfactory.

When analyzing the results of the survey on the scale of anxiety and depression HADS, it was found that 72% of the students examined by us did not have significantly expressed symptoms of anxiety, and 78.4% had significantly expressed symptoms of depression (see Table 4 herein).

Table 4
Levels of symptoms of anxiety and depression in students according to the HADS scale (%)

<table>
<thead>
<tr>
<th>Levels</th>
<th>Signs of anxiety</th>
<th>Signs of depression</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males Females</td>
<td>Total</td>
</tr>
<tr>
<td>No significant symptoms</td>
<td>77.8</td>
<td>69</td>
</tr>
<tr>
<td>Subclinical anxiety/depression</td>
<td>14.6</td>
<td>17.1</td>
</tr>
<tr>
<td>Clinically significant anxiety/depression</td>
<td>7.6</td>
<td>13.9</td>
</tr>
</tbody>
</table>

The incidence rate of anxiety among the examined students was recorded to be 29% and depression 21.6%. Moreover, signs of subclinically expressed anxiety were found in 16.2% of students, subclinically expressed depression was detected in 15%, clinically expressed anxiety in 11.8% and clinically expressed depression in 6.6%. The gender differences did not reach the level of statistical significance. Some differences in the prevalence of signs of anxiety and depression depending on marital status, housing conditions, place of residence, source of income, academic performance, employment status, sleep duration, smoking, alcohol consumption and LPA are presented in Table 5.

In the population surveyed by us, the prevalence of subclinical depression was higher among the students who smoke compared with non-smokers \( (p=0.0032) \) and among students with LPA compared with students with the normal level of physical activity \( (p=0.0001) \). Clinically expressed depression was more common among students with higher education compared with peers with secondary education \( (p=0.0258) \); in the group of students who rated their living conditions as good, compared with peers who rate their living conditions as excellent \( (p=0.028) \); among students with debts compared with those without them \( (p=0.0001) \); among employed students compared with unemployed \( (p=0.0001) \); in students with LPA compared with peers with a normal level of physical activity \( (p=0.0001) \).

In the context of the Covid-19 pandemic, the mental health symptoms are a growing concern around the world. According to a survey of 18,764 students from 14 campuses, conducted from late March to May 2020, more than 50% of American college students are concerned about contracting Covid-19, and about 90% about their personal safety [14]. Students also had a lower level of psychological well-being than before the pandemic [14]. Studies conducted in Chinese colleges show that students are concerned about delays in studies, negative economic consequences, routines and daily activities, and the prevalence of anxiety disorders is 24.9% [5]. Studies of anxiety and depressive symptoms among students showed its high prevalence in Turkey (45%) and Cyprus (anxiety level 64.1% and depression 57.3%) [6,7]. The prevalence of stress among students of medical faculties of the University of Zaragoza (Spain) almost a year after the start of the pandemic was 13.1%, anxiety 71.4% and depression 81%. The presence of stress and anxiety among university students due to Covid-19 almost a year after the start of the pandemic, according to researchers, confirms that psychological support measures for these groups should be the priority [8]. Differences in the prevalence of anxiety and depressive symptoms in different countries may be associated with different rates of the spread of the virus in these countries and various anti-epidemic and social measures.

To assess the impact of high anxiety, the level of anxiety and depression on the indicators included in the study, we conducted a correlation analysis. As presented in Table 6 herein, statistically significant correlations were found between PA and housing conditions, employment, main source of income, preparation time for classes, number of cigarettes smoked, alcohol consumption, rate of alcohol consumption, sports, physical exercises or active leisure activities of high intensity; between SA and housing conditions, sleep duration, academic performance, rate of alcohol consumption.
Table 5
Levels of anxiety and depression depending on the students activity indicators (%)

<table>
<thead>
<tr>
<th>Indicators</th>
<th>Anxiety Subclinical level</th>
<th>Anxiety Clinical level</th>
<th>Depression Subclinical level</th>
<th>Depression Clinical level</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No signs</td>
<td>Anxiety</td>
<td>No signs</td>
<td>Depression</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Subclinical level</td>
<td></td>
</tr>
<tr>
<td>Marital status</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>married</td>
<td>96.6</td>
<td>0</td>
<td>3.4</td>
<td>79.3</td>
</tr>
<tr>
<td>not married</td>
<td>70.2</td>
<td>17.4</td>
<td>12.4</td>
<td>78.3</td>
</tr>
<tr>
<td>Education</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>higher</td>
<td>64.1</td>
<td>15.4</td>
<td>20.5</td>
<td>59</td>
</tr>
<tr>
<td>Vocational secondary</td>
<td>50</td>
<td>37.5</td>
<td>12.5</td>
<td>87.5</td>
</tr>
<tr>
<td>secondary</td>
<td>73.3</td>
<td>15.9</td>
<td>10.8</td>
<td>78.5</td>
</tr>
<tr>
<td>Type of residence</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>dormitory</td>
<td>72</td>
<td>12</td>
<td>16</td>
<td>80</td>
</tr>
<tr>
<td>Own apartment</td>
<td>67.8</td>
<td>17.7</td>
<td>14.5</td>
<td>71</td>
</tr>
<tr>
<td>With parents</td>
<td>73.6</td>
<td>16.2</td>
<td>10.2</td>
<td>80.3</td>
</tr>
<tr>
<td>Rental apartment</td>
<td>70.2</td>
<td>16.7</td>
<td>13.1</td>
<td>77.4</td>
</tr>
<tr>
<td>Housing conditions</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>excellent</td>
<td>74.3</td>
<td>15.9</td>
<td>9.8</td>
<td>81.5</td>
</tr>
<tr>
<td>good</td>
<td>69</td>
<td>15.9</td>
<td>15.1</td>
<td>73</td>
</tr>
<tr>
<td>satisfactory</td>
<td>63.6</td>
<td>22.7</td>
<td>13.6</td>
<td>72.7</td>
</tr>
<tr>
<td>Place of residence</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rural</td>
<td>75.3</td>
<td>18.8</td>
<td>5.9</td>
<td>77.2</td>
</tr>
<tr>
<td>urban</td>
<td>71</td>
<td>15.4</td>
<td>13.6</td>
<td>78.7</td>
</tr>
<tr>
<td>Source of income</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>parents' funds</td>
<td>69.9</td>
<td>17.6</td>
<td>12.5</td>
<td>80.4</td>
</tr>
<tr>
<td>Own income</td>
<td>74.2</td>
<td>15.2</td>
<td>10.6</td>
<td>66.7</td>
</tr>
<tr>
<td>Other income</td>
<td>95.7</td>
<td>0</td>
<td>4.3</td>
<td>82.6</td>
</tr>
<tr>
<td>Academic performance</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>excellent</td>
<td>71</td>
<td>13.2</td>
<td>15.8</td>
<td>78.9</td>
</tr>
<tr>
<td>good</td>
<td>71.2</td>
<td>17.4</td>
<td>11.4</td>
<td>78.2</td>
</tr>
<tr>
<td>satisfactory</td>
<td>76.5</td>
<td>14.7</td>
<td>8.8</td>
<td>79.4</td>
</tr>
<tr>
<td>Academic debts</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>yes</td>
<td>68.8</td>
<td>15.1</td>
<td>16.1</td>
<td>74.2</td>
</tr>
<tr>
<td>no</td>
<td>72.9</td>
<td>16.6</td>
<td>10.5</td>
<td>83.5</td>
</tr>
<tr>
<td>Employment status</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>employed</td>
<td>73.7</td>
<td>15.8</td>
<td>10.5</td>
<td>68.4</td>
</tr>
<tr>
<td>unemployed</td>
<td>71.7</td>
<td>16.3</td>
<td>12</td>
<td>79.9</td>
</tr>
<tr>
<td>Night shifts</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>yes</td>
<td>78.3</td>
<td>13</td>
<td>8.7</td>
<td>82.6</td>
</tr>
<tr>
<td>no</td>
<td>70.5</td>
<td>17.7</td>
<td>11.8</td>
<td>58.8</td>
</tr>
<tr>
<td>Sleep duration</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Less than 8 h</td>
<td>68.5</td>
<td>18</td>
<td>13.5</td>
<td>77.4</td>
</tr>
<tr>
<td>8 h and more</td>
<td>78</td>
<td>13.2</td>
<td>8.8</td>
<td>79.9</td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>yes</td>
<td>56</td>
<td>24</td>
<td>20</td>
<td>48</td>
</tr>
<tr>
<td>no</td>
<td>73</td>
<td>15.8</td>
<td>11.2</td>
<td>80.2</td>
</tr>
<tr>
<td>Alcohol</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>yes</td>
<td>68.9</td>
<td>18</td>
<td>13.1</td>
<td>65.6</td>
</tr>
<tr>
<td>no</td>
<td>72.5</td>
<td>15.9</td>
<td>11.6</td>
<td>80.5</td>
</tr>
<tr>
<td>Low physical activity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>yes</td>
<td>66.7</td>
<td>21.6</td>
<td>11.7</td>
<td>63.4</td>
</tr>
<tr>
<td>no</td>
<td>72</td>
<td>16.9</td>
<td>11.1</td>
<td>93.8</td>
</tr>
</tbody>
</table>

Notes: differences are statistically significant between students, *smokers and non-smokers (p = 0.0032); **with low and normal physical activity (p = 0.0001); ***with higher and secondary education (p = 0.0258); ****estimating their living conditions as good and excellent (p = 0.028); *****who have and do not have debts (p = 0.0001); ******employed and unemployed (p = 0.0001).

Table 6
Correlations between the levels of anxiety and depression with some of the studied indicators (p<0.05)

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Anxiety</th>
<th>Depression</th>
<th>Personal anxiety</th>
<th>Situational anxiety</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sleep duration</td>
<td>0.16</td>
<td>-</td>
<td>-</td>
<td>-0.10</td>
</tr>
<tr>
<td>Housing conditions</td>
<td>0.11</td>
<td>0.17</td>
<td>0.11</td>
<td>0.14</td>
</tr>
<tr>
<td>Employment</td>
<td>-</td>
<td>-</td>
<td>0.12</td>
<td>-</td>
</tr>
</tbody>
</table>

116 | Cardiometry | Issue 21. February 2022
Statistically significant correlations were also detected between the level of anxiety and housing conditions, main source of income, sleep duration, number of cigarettes smoked, smoker’s index, volume of fortified wine usually consumed per day; between the level of depression and living conditions, current smoking and the number of days per week of sports, exercise or moderate-intensity leisure activities. Despite the fact that correlations do not indicate a causal relationship, we can talk about the possibility of the influence of the factors we identified on the levels of anxiety and depression. Relationships between anxiety and depressive symptoms and employment status, factors of educational activity have been identified in other studies [8,13]. Some of these factors are quite flexible and easily controllable. Rational modulation and control of them can reduce the level of anxiety, as well as manifestations of anxiety and depression. Recommendations on the organization of sports or physical exercises, on the rational distribution of time for preparing for classes and ensuring sufficient sleep, quitting smoking and drinking alcohol for the students examined by us can be useful.

**Conclusion**

Thus, in the surveyed population of students a year after the onset of the Covid-19 pandemic, an unfavorable situation was revealed in terms of the prevalence of anxiety and depressive symptoms. It has been identified that the prevalence of high levels of PA and moderate levels of SA among females is higher than among males. High levels of SA were more common among students with higher education, living in rented apartments, in rural areas and having debts, and high levels of PA in the group of students who assess their living conditions as satisfactory. Clinically pronounced depression was more common among students with higher education, LPA, who assess their living conditions as good, have debts and combine study with work. Associative relationships were detected between the levels of SA, PA, anxiety, depression and parameters of students’ everyday activity included in the study. The results obtained by us can be used to improve the academic process, as well as to preserve the mental health of students at KBSU.

**Statement on ethical issues**

Research involving people and/or animals is in full compliance with current national and international ethical standards.

**Conflict of interest**

None declared.

**Author contributions**

The authors read the ICMJE criteria for authorship and approved the final manuscript.
References


Anatomical and topographical features of the gracilis muscle of the thigh from the position of using it for autotransplantation


1Almazov National Medical Research Centre, 197341, Russia, St. Petersburg, 2 Akkuratova street
2National Medical Research Center of Oncology named after N.N.Petrov, 197758, Russia, St. Petersburg, Leningradskaya str., 68
3North-Western State Medical University named after I.I. Mechnikov, 195067, Russia, St. Petersburg, Piskarevskij prospect, 47

*Corresponding author: spb.gda@yandex.ru

Abstract

Autotransplantation of a free flap of the gracilis muscle is currently actively used in reconstructive surgery for persistent prosoplegia, injuries of the brachial plexus, for plastic closure of defects in the upper lip, and in the treatment of pelvic sepsis. This study provides important information about the anatomical features of the gracilis muscle and its neurovascular bundle, which are highly variable.

The study of the anatomical and topographic features of this fine thigh muscle was carried out on a sectional study of 25 corpses of both sexes, 50 lower limbs. The present study demonstrates that the median value of the total length of m. Gracilis, the length of its muscular part, as well as the length of the tendon were 452.25 (439.7; 462.0); 225.3(208.1;239.0); 230.5 (213.0; 244.4) mm, respectively. The number of vascular pedicles included in m. Gracilis, ranged from 1 to 5. In 86% of the cases, the deep femoral artery formed the dominant vascular pedicle, and in 14% of cases, it was the medial circumflex artery. Small secondary vascular pedicles originated from the descending genicular artery or the anterior branch of the obturator arter: from the basin of the internal iliac artery. The length of the main feeding artery varied from 76 to 134 mm, the median value was 100.5(90;117) mm, and its diameter ranged from 1.4 to 2.1 mm (M = 1.9(1.8;2, 0) mm.

The innervation of the fine muscle of the thigh was carried out by the anterior branch of the obturator nerve, which in 82% of the cases was represented by a single trunk; less often a loose type of structure was observed. The proper nerve was located at a distance of 108.5 (96; 117) mm from the origin of the muscle; its diameter was 2.1 (1.9; 2.2) mm.

Knowledge of the topographic and anatomical relationships of the neurovascular bundle with the surrounding formations, variants of its individual anatomical variability are the key to successful reconstructive surgery.

Keywords

Thigh gracilis muscle, Vascular pedicle, Obturator nerve, Transplantation

Introduction

Transplantation of a free flap of the gracilis muscle is increasingly used in modern reconstructive surgery. This includes prosoplegia of the facial muscles, lesions of the brachial plexus, plastic closure of upper lip defects, and even the treatment of pelvic sepsis. Usually, the surgical technique includes harvesting of the flap with its neurovascular bundle, revascularization using microvascular anastomoses, and reinnervation in order for the transferred gracilis muscle to become functionally significant. Thorough knowledge by the surgeon of the topographic and anatomical relationships of the neurovascular bundle with the surrounding formations and its variants of individual anatomical variability are the key to successful surgical intervention.

M. Gracilis is a thin long muscle located in the medial thigh most superficially relative to other adductor muscles. Its shape is flattened, wide at the top and gradually tapering downwards. It originates from the lower branch of the pubic and adjacent part of the ischial bones, and its tendon connects to the tendons of the sartorius and semitendinosus muscles and is attached to the upper part of the tibia medial to its tuberosity. The thin thigh muscle performs an auxiliary function in hip adduction and flexion, knee flexion, internal rotation of the knee, and therefore can be used as a donor without significant motor deficit,
which is quickly and easily compensated by agonist muscles. On the other hand, such characteristics of M. Gracilis as size, shape, length, reliable blood supply to the dominant vascular pedicle provide a good cosmetic result, forming an adequate facial contour, and its innervation by the motor obturator nerve, acceptable muscle strength with a good excursion allow us to hope for a satisfactory functional rehabilitation [1-4]. However, to date, a number of unresolved problems remain associated with impaired blood supply to the transplanted muscle flap, a feature of modeling the muscle flap, “donor zone disease”. A more in-depth study of the anatomical and topographic features of m. Gracilis of its neurovascular bundle will obviously improve the results of surgical treatment of patients with persistent prosoplegia.

Materials and methods

The study of the variability in the structure of the small muscle of the thigh was carried out on a sectional study of 25 corpses of both sexes, 50 lower limbs (12 males and 13 females) on the basis of the patho-anatomical department of the V.A. Almazov National Medical Research Centre of the Ministry of Health of Russia. Criteria for inclusion in the study were the deceased without trauma, tumor, deformities of the medial surface of the thigh; diseases of the musculoskeletal system; age of the dead is over 18; prescription of death is not more than 24-36 hours. The bodies of the dead before the study were stored under the same conditions, in a refrigerator at a temperature of +2°C.

Our study was carried out using the standard thigh incisions to search for deep vein thrombi of the thigh. Leg length was measured from the anterior superior iliac spine to the lateral malleolus. The skin on the medial surface of the thigh was dissected, and the thin muscle of the thigh was exposed. The muscle, its vessels and nerve were separated from the surrounding tissues along the entire length. Total length of m. Gracilis was measured from its origin (inferior ramus of the pubis) to its insertion (upper part of the medial surface of the tibia). The width and thickness of the muscle, the length of its muscular and tendon parts were measured. The number of vascular pedicles, the entrance point of each of the vascular pedicles and their sources, the length and diameter of the vessels, the nerve in the dominant vascular pedicle, and the number of nerve branches were also studied. The study was approved by the Ethics Committee of the Federal State Budgetary Institution “V.A. Almazov Almazov National Medical Research Centre” of the Ministry of Health of Russia, Extract No. 03012020 from Record No. 1-20 dated January 20, 2020 prepared by the Ethics Committee meeting.

According to the results of measuring the parameters of m.Gracilis, distribution series were compiled. For each variation series, the median value and interquartile range were calculated. In the process of primary statistical data processing, the Kolmogorov-Smirnov criterion was less than 0.05, and due to that fact, non-parametric statistical methods were used for the non-normal distribution of data. Spearman's coefficient was used to assess the relationship between the parameters represented by quantitative data. Qualitative characteristics of the tightness of the relationship of the rank correlation coefficient were assessed on the Chaddock scale. The indicators were considered statistically significant at p<0.05.

Results

The median length of the lower limbs was 904.4(871.1;930.0) mm. The corresponding value of the total length of m. Gracilis was 452.25(439.7;462.0) mm, the median value of the length of the muscular part was 225.3(208.1;239.0) mm, and the median value of the length of the tendon was 230.5(213.0;244.4) mm. The median width in the belly of the gracilis muscle in its widest part was 34.5 (29.2; 37.5) mm. The median thickness of the belly and tendon was 7.3(6.8;8.0), 2.6(2.3;2.8) mm, respectively (Table 1). In this case, there was a direct noticeable correlation between the lengths of m. Gracilis and the lower limb (R=0.64, p=0.019). Additionally, a moderate statistically significant relationship was found between the width of the

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Median (Q1;Q3) mm</th>
<th>Min (mm)</th>
<th>Max (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lower limb length</td>
<td>904.4(871.1;930.0)</td>
<td>714</td>
<td>1008.2</td>
</tr>
<tr>
<td>m. Gracilis length</td>
<td>452.25(439.7;462.0)</td>
<td>416</td>
<td>501.6</td>
</tr>
<tr>
<td>Muscle length</td>
<td>225.3(208.1;239.0)</td>
<td>179</td>
<td>288.1</td>
</tr>
<tr>
<td>Tendon length</td>
<td>230.5(213.0;244.4)</td>
<td>184.3</td>
<td>260.7</td>
</tr>
<tr>
<td>Belly width of the gracilis femoris</td>
<td>34.5(29.2;37.5)</td>
<td>19</td>
<td>50</td>
</tr>
<tr>
<td>Muscle thickness</td>
<td>7.3(6.8;8.0)</td>
<td>5.4</td>
<td>9.8</td>
</tr>
<tr>
<td>Tendon thickness</td>
<td>2.6(2.3;2.8)</td>
<td>1.5</td>
<td>3.1</td>
</tr>
</tbody>
</table>

Table 1
Morphometric characteristics of the gracilis muscle of the thigh (M. Gracilis)
useful muscle part of the graft (R=0.32, p=0.022), the width of the distal part of the tender muscle (R=0.33, p=0.017) and the length of the lower limb.

The number of vascular pedicles included in m. Gracilis, ranged from 1 to 5. Most often, in 46% of the cases, there was one main vascular pedicle, less often, in 34% of the cases, there was a variant with one additional pedicle. In 14% of the studied cases, the main vascular pedicle with two additional ones was identified. Three additional vascular pedicles were found in 4% of the cases. Only in 2% of the cases there were four additional vascular pedicles.

The distance from the origin of m. Gracilis to the point of entrance of the main vascular pedicle into the muscle ranged from 78 to 130 mm. The length of the main feeding artery varied from 76 to 134 mm, the median value was 100.5(90;110) mm, and its diameter was from 1.4 to 2.1 mm (M = 1.9(1.8;2.0) mm The artery was most often accompanied by two veins (see Table 2 herein).

Table 2
Morphometric characteristics of the neurovascular bundle of the fine muscle of the thigh (M. Gracilis)

<table>
<thead>
<tr>
<th>Morphometric parameters</th>
<th>Median (Q1;Q3) mm</th>
<th>Min (mm)</th>
<th>Max (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distance to the entry of the main vascular pedicle</td>
<td>100.5(90;110)</td>
<td>78</td>
<td>130</td>
</tr>
<tr>
<td>Length of the main feeding artery</td>
<td>109(98;119)</td>
<td>76</td>
<td>134</td>
</tr>
<tr>
<td>Main feeding artery diameter</td>
<td>1.9(1,8,2,0)</td>
<td>1.4</td>
<td>2.1</td>
</tr>
<tr>
<td>Nerve length</td>
<td>108.5(96;117)</td>
<td>76</td>
<td>130</td>
</tr>
<tr>
<td>Nerve diameter</td>
<td>2.1(1,9,2,2)</td>
<td>1.5</td>
<td>3</td>
</tr>
</tbody>
</table>

In 86% of the cases, the deep femoral artery formed the dominant vascular pedicle. In 14% of the cases, this function was performed by its branch, the medial artery that circumflexed the femur. Small secondary vascular pedicles originated from the anterior branch of the obturator artery or descending genicular artery. The length of additional feeding vessels was measured from the lower branch of the pubic bone and varied from 58 mm to 291 mm, depending on the number of vascular bundles and the variant of their discharge (Table 3).

According to our data, the proper nerve was located at a distance of 108.5 (96; 117) mm from the origin of the muscle; its diameter was 2.1 (1.9; 2.2) mm.

In 41 preparations (82% of the cases) the indicated nerve was represented by one main trunk, in 5 cases the nerve was represented by 2 trunks (10%), and in 4 cases the loose type of nerve structure was recorded (8%).

In 86% of the cases, the deep femoral artery formed the dominant vascular pedicle. In 14% of the cases, this function was performed by its branch, the medial artery that circumflexed the femur. Small secondary vascular pedicles originated from the anterior branch of the obturator artery or descending genicular artery. The length of additional feeding vessels was measured from the lower branch of the pubic bone and varied from 58 mm to 291 mm, depending on the number of vascular bundles and the variant of their discharge (Table 3).

According to our data, the proper nerve was located at a distance of 108.5 (96; 117) mm from the origin of the muscle; its diameter was 2.1 (1.9; 2.2) mm.

In 41 preparations (82% of the cases) the indicated nerve was represented by one main trunk, in 5 cases the nerve was represented by 2 trunks (10%), and in 4 cases the loose type of nerve structure was recorded (8%).

Table 3
The length of the vessels that feed m. Gracilis of the thigh

<table>
<thead>
<tr>
<th>Vascular pedicle</th>
<th>Distance from the inferior ramus of the pubis Median (Q1;Q3) mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Main pedicle</td>
<td>100.5(90;110) mm</td>
</tr>
<tr>
<td>1st additional pedicle</td>
<td>204 (189;210) mm</td>
</tr>
<tr>
<td>2nd additional pedicle</td>
<td>272 (250;288) mm</td>
</tr>
<tr>
<td>3rd additional pedicle</td>
<td>270 (270;291) mm</td>
</tr>
<tr>
<td>4th additional pedicle</td>
<td>58 mm</td>
</tr>
</tbody>
</table>

Discussion

Thin muscle is widely used in reconstructive surgery. Previously, anatomical and radiological studies were carried out regarding the parameters of m. Gracilis and its vascular pedicles [5-7], which were focused on the length of the tendon part, due to purely applied tasks, i.e. the use of the muscle in rehabilitation operations for post-traumatic plexopathies. Only a few of them mentioned the muscle part used in the rehabilitation of mimic muscle paresis [9-14, 16,17].

An analysis of the literature data shows that the average width of the muscle part of m. Gracilis at its base, in the middle and at the end is 34.5±6.395, 26.3±4.682, 17.4±3.527 mm, respectively. At the same time, Dziedzic DW , et.al [8] and Rajeshwari MS, Roshankumar BN indicated the width of the belly of m. Gracilis 31.9 and 39 mm only, respectively [15].

The present study demonstrates that the median value of the total length of m. Gracilis, the length of its muscular part, as well as the length of the tendon are 452.25 (439.7; 462.0); 225.3(208.1;239.0); 230.5 (213.0; 244.4) mm, respectively.

In this case, there is a direct correlation between the lengths of m. Gracilis and the lower extremity, which is in full agreement with the results of the study.
by Limitlaohaphan C et al. and Chiang et al. [18-21]. Additionally, a statistically significant relationship was found between the width of the useful muscle part of the graft, the width of the distal part of the tender muscle, and the length of the lower limb.

In the present study, attention was also focused on the study of the neurovascular pedicle of m. Gracilis. Its blood supply is variable and can be carried out from the branches of the femoral artery: the deep artery of the thigh, its branches - the medial artery, the circumflex femur, the descending knee artery, and also from the pool of the internal iliac artery - the anterior branch of the obturator artery [1-3].

Usually the above mentioned muscle of the thigh has two or three vascular pedicles penetrating into it from the inner surface. The proximal pedicle is dominant, enters at the junction of the upper and middle thirds of the muscle and is the main source of its blood supply, includes an artery, two draining veins, and the anterior branch of the obturator nerve. The medial circumflex femoral artery and the deep femoral artery usually form the proximal dominant vascular pedicle. The number of small secondary pedicles varies from one to five, and they include only vessels (artery and veins) and supply blood to the distal and smaller part of the muscle [3]. The small pedicle may include the anterior branch of the obturator artery, the descending genicular artery.

Our study showed that the number of vascular pedicles included in m. Gracilis, varied from 1 to 5, namely, in 46% of the cases there was one pedicle, in 34% of the cases we detected two pedicles, in 14% we revealed three pedicles, in 4% of the cases there were four pedicles, and extremely rarely - in 2% of the cases, the blood supply to the muscle was carried out according to the loose type and has at least five vascular pedicles. Most often (63.24%), the main vascular pedicle was located at a distance of 88-112 mm from the origin of m. Gracilis. These results were approximately the same as obtained in the observations of Rajeshwari MS, Roshankumar BN [15] and Vigato E et.al. [5], in which they mentioned that their number was 1-5 (most 1-3). The main pedicle enters the muscle at a distance of 100.5(90;110) mm. The diameter of the neurovascular pedicle at their entrance ranges from 1.4 to 2.1 mm, accompanied by two veins, which is somewhat larger than in the study of Vigato E et.al., the average value is 1.7 [5].

In this study, in all cases, the donor muscle was innervated by a branch of the obturator nerve. According to the reference literature, it is formed from L2-L4-spinal roots and, upon exiting their retroperitoneal space, bypassing the obturator canal, is divided into terminal posterior and anterior branches, the latter, having passed between the long and short adductor muscles, innervates the fine muscle of the thigh. Despite the fact that in most cases (82% of cases) this nerve is represented by one main trunk, loose types of its structure were also observed.

Conclusions
The anatomical structure of the neurovascular bundle of M. Gracilis is highly variable, however even the most extreme cases of individual anatomical variability it cannot exclude latter from being used as a donor in reconstructive surgery. This study provides important actual information about the anatomical features of m. Gracilis and can be useful in practice at the stage of preoperative planning.

Statement on ethical issues
Research involving people and/or animals is in full compliance with current national and international ethical standards.

Conflict of interest
None declared.

Author contributions
The authors read the ICMJE criteria for authorship and approved the final manuscript.

References
Functional state of the students’ cardiovascular system under the conditions of mountain hypoxia

Vakha A. Anzorov*, Svetlana V. Moryakina

Chechen State University named after A.A. Kadyrov, 364024, Russia, Grozny, Sheripova str., 32

*Corresponding author: vaha-anzorov@mail.ru

Abstract
The article presents the functional state of the cardiovascular system of students under conditions of high-altitude hypoxia. From the analysis of the results of our research, it can be argued that due to the fact that students live under conditions of a reduced oxygen content in the atmospheric air, there is a significant increase in the systolic output of their hearts. Thus, the systolic blood volume of students under the conditions of mountain hypoxia increases, reaching the maximum values of 63.4 ml (P <0.05) in males and 64.4 in females in the middle-level mountains. The range of pulse pressure between the groups of males is 1.5%, and females 1.7. The level of average dynamic blood pressure in males in the middle mountains is 3.5% lower, and in females of the same age is 3.9% lower, respectively, compared to the plains. The drop in the minute blood volume at an altitude of 1600 m among female students was 2.1%, and among peers - 2.2, compared to the 170 m level. The decrease in total peripheral vascular resistance (TPVR) at the maximum altitude was 1.5% in females, and 2.7% in males, compared to the plain-related data. The value of blood circulation efficiency coefficient (CEC) and endurance factor (EF) in the middle-level mountains among female students decreased by 6.2 and 2.7%, among peers - by 4.2 and 5.4%, compared to the plain. Thus, the depth of the changes caused is directly proportional to the level of the acting hypoxia.

Keywords
Student, Hypoxia, Systolic blood volume, Coefficient of blood circulation efficiency, Coefficient of endurance

Imprint

Up to 40% of the Earth’s land is formed by mountains, and up to 600 million people live there. Since ancient times, people has been drawn to the mountains and this is due to their attractiveness, recalcitrance, searching for new sources of energy, development of subsoil rich in natural resources, development of mountain sports, erection of sports and recreation facilities.

A distinctive feature of mountain regions is the diversity of their natural and climatic conditions. One of the main factors affecting the systems of the human body in the mountains is the insufficient content of oxygen in the atmospheric air. Human activity under the influence of adverse environmental factors requires increasing stress of the body systems.

Therefore, in physiology, great attention is paid to the problem of adaptation to high-altitude hypoxia. After the organism enters the mountainous area, adaptive changes to the new environmental conditions occur in it.

Under the mountain conditions, a low partial pressure of oxygen can lead to disruption of the functional activity of vital body systems. This, in turn, is due to insufficient energy supply to the cells of the body due to a violation of metabolic processes in them. To overcome the negative effects of the environment, a complex of adaptive reactions is launched by the body, which leads to the normalization of the process of external respiration, improves the diffusion capacity of the lung tissue and reduces the heart rate [17]. An important problem of our time is to increase the efficiency of the functional activity of the body systems, as this determines the state of health and the performance of the body as a whole. The consequence of the adaptive reactions of the body to the effects of hypoxia is the stimulation of the body’s systems and its enhanced performance that is widely used in medicine and sports physiology.

Hypoxia therapy improves the general condition of the body, normalizes all types of metabolism, enhances blood circulation, metabolism and increases the body’s resistance to adverse factors [5, 8]. Mountain hypoxia was widely used in ancient times to increase the functional reserves of the body [3].
The higher is the level of the body’s performance, the better are the functional abilities of the body’s systems that provide it with oxygen, which include the systems of respiration and blood circulation [6]. According to many researchers, the most effective method of exposure to hypoxia is to stay in the mountains [7, 11, 13, 14]. Moreover, at the initial stage of the influence of the mountain climate, the body’s working capacity decreases [9]. This is accompanied by an unusual increase in the function of the respiratory and circulatory systems.

According to some authors, the effect of intermittent hypoxia is highly effective for improving the performance [1, 2, 4, 12].

A feature of the population living in mountainous areas is the presence of good health and high efficiency in their activities, which persist until their old age [16]. Despite the numerous studies conducted to study the effect of hypoxia made on the body, they are mainly devoted to the level of the human performance, but not the state of functional systems.

Due to the fact that a significant part of the population lives in the mountainous regions of the Chechen Republic, it is important to study the effect of high-altitude hypoxia on the functional state of the cardiovascular system.

Materials and methods

The experimental material was collected in the laboratories of the Department of Physiology and Anatomy of Humans and Animals. 150 (75 females and males) students were involved into our research.

The age of the test subjects ranged from 19 to 21 years. According to the principle of analogues (by age, weight and gender), depending on the height of their residence above sea level (170 m - Grozny, 600 m - Shatoysky district and 1600 m - Sharovsky district) were divided into three groups. Each group consisted of 25 students.

To analyze the functional state of the cardiovascular system, we used the rhythm of heart contraction, blood pressure and some calculated indicators. Biometric checking of the results of the experiment was carried out using the program “Biostatistics”.

Results and Discussion

The state of the cardiovascular system of students under hypoxia conditions is shown in Tables 1, 2 and in Figure 1 herein.

| Table 1 | Cardiovascular system of young males |
| Altitude above sea level, meters | Systolic blood pressure, mm Hg | Diastolic blood pressure, mm Hg | Heart rate, beats per minute |
| 170 | 120.4±3.50 | 80.6±2.03 | 73.2±2.29 |
| 600 | 119.6±3.38 | 79.6±2.06 | 71.8±2.08 |
| 1600 | 117.2±3.29 | 77.1±2.18 | 69.6±2.50 |

| Table 2 | Indicators of the cardiovascular system of female students |
| Altitude above sea level, meters | Systolic blood pressure, mm Hg | Diastolic blood pressure, mm Hg | Heart rate, beats per minute |
| 170 | 119.7±3.32 | 78.6±2.32 | 75.8±3.15 |
| 600 | 117.9±3.29 | 77.0±2.77 | 74.4±2.94 |
| 1600 | 115.5±3.14 | 75.1±2.85 | 72.2±3.07 |

Figure 1. The effect of hypoxia on the parameters of the cardiovascular system

The level of the studied indicators under conditions of insufficient oxygen content in the inhaled air shows a gradual slight decrease.

Thus, the drop in the maximum blood pressure in young males at an altitude of 600 meters above sea level is 0.8 mm Hg, 1600 - 3.2 mm Hg, and for females 1.8 and 4.2, respectively, compared to the plain-related data - 120.4 and 119.7.

Under conditions of lowlands and middle mountains, diastolic blood pressure in females is lower by 1.6 and 3.5 mm Hg, in peers - 1.4 and 3.5 mm Hg, than on the plains.
At an altitude of 600 and 1600 meters, the heart rate in young males is less than 1.4 and 3.6 beats per minute, and in peers - 1.4 and 3.6, respectively, than at the level of 170 meters.

Apparently, the drop in heart rate is due to an increase in the excitability of the vagus nerve.

Our colleagues came to similar conclusions [6, 19].

The lack of oxygen in the inhaled air contributes to an increase in the weight and size of the adrenal glands, although deepening of hypoxia leads to the suppression of their functional activity, according to [18].

The results of the conducted studies are shown in Tables 3-5 and in Figures 2-3 herein. They show that the only of the studied indicators, which undergoes a significant change when exposed to high-altitude hypoxia in students, is the systolic blood volume.

**Table 3**
The functional state of the cardiovascular system of young males

<table>
<thead>
<tr>
<th>Altitude above sea level, meters</th>
<th>Pulse pressure (PP), mm Hg</th>
<th>Average dynamic blood pressure (BP av.dyn.), mm Hg</th>
<th>Systolic blood volume (SBV), ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>170</td>
<td>39.8±1.47</td>
<td>97.3±2.65</td>
<td>60.9±0.43</td>
</tr>
<tr>
<td>600</td>
<td>40.4±1.41</td>
<td>96.2±2.60</td>
<td>61.8±0.43</td>
</tr>
<tr>
<td>1600</td>
<td>40.1±1.15</td>
<td>93.9±2.65</td>
<td>63.4±0.55*</td>
</tr>
</tbody>
</table>

* - P < 0.05

**Table 4**
The level of indicators in young males

<table>
<thead>
<tr>
<th>Terrain</th>
<th>Indicators</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Minute blood volume (MBV), l/min</td>
</tr>
<tr>
<td></td>
<td>Total peripheral vascular resistance (TPVR), dyn·s·cm⁻⁵</td>
</tr>
<tr>
<td></td>
<td>Circulation Efficiency Coefficient (CEC)</td>
</tr>
<tr>
<td></td>
<td>Endurance Factor (EF)</td>
</tr>
<tr>
<td>Plain</td>
<td>4.5±0.11</td>
</tr>
<tr>
<td>Lowlands</td>
<td>4.4±0.10</td>
</tr>
<tr>
<td>Middle mountains</td>
<td>4.4±0.12</td>
</tr>
</tbody>
</table>

The level of pulse pressure in young males of different groups ranged from 39.8 mm Hg on the plain up to 40.4 in the lowlands, and in females from 40.4 mm Hg at an altitude of 1600 meters to 41.1 on the plain. The given values of PP correspond to the norm. The value of the average dynamic pressure of students with increasing altitude demonstrated a gradual decrease.

So, in the middle mountains, its level is lower by 3.4 mm Hg in males and 3.7 in females compared to the plain-related data.

The average level of blood pressure in students in the plains and in young males in the middle mountains is above the upper limit of the norm.

The amount of blood ejected into the arterial system by the ventricles of the heart per contraction in
young males living at an altitude of 1600 meters is 2.5 ml higher (P <0.05), and in female students 1.6 ml higher than those recorded on the plain. The SBV value is within the normal range. The minute blood volume in students under the influence of hypoxia slightly decreases. Thus, the decrease in the level of MBV in young men and their peers in the middle mountains is 0.1 l/min, compared to the plain. For all groups of students, the value of the TPVR is below the norm.

The value of the total peripheral vascular resistance decreases with the deepening of hypoxia. Its level at a height of 600 meters is higher for female students and their peers by 15 and 52 dyn·s·cm$^{-5}$, and 1600 - 24 and 46, respectively, than it is the case with the level of 170 meters. The average values of TPVR by groups remain within the norm.

The decrease in CEC in males and females in the lowlands is 13 and 76, in the middle mountains 124 and 194, respectively, as compared to the plains. The impact of mountain hypoxia leads to a slight decrease in the endurance factor of students. Its fall at an altitude of 1600 meters is 0.5 for female students and 0.2 for their peers versus the data recorded on the plain.

The average levels of CEC and EF are above the upper limit of the norm, which indicates the presence of fatigue and insufficient fitness of the cardiovascular system. The beginning of adaptation to the lack of oxygen in the inhaled air is accompanied by an increase in the heart rate and minute volume of blood [20].

The minute volume of blood increases due to a rise in the heart rate and systolic blood ejection. With prolonged exposure to a reduced oxygen content in the atmospheric air, their level decreases, even below the initial values [10]. Moreover, the constant increase in the study load increases the mental load of students, which reduces their physical activity. This, in turn, inhibits the functional activity of body systems, including the cardiovascular system.

The low level of physical fitness and poor health of students are reported [15]. In this connection, in order to improve their health, it is necessary to increase the role of physical exercises.

Under the influence of oxygen deficiency in the atmospheric air, only the systolic blood volume shows significant changes among the indicators of the functional state of the cardiovascular system.

**Conclusions**

1. The systolic blood volume is the only indicator of the students’ cardiovascular system functional state that demonstrates significant changes under the influence of high-altitude hypoxia.

2. The difference between the maximum and minimum values of pulse pressure between the groups of males and females has been reported to be 0.6 and 0.7 mm Hg, respectively.

3. The value of the average dynamic pressure in young males at an altitude of 1600 meters is lower by 3.4 mm Hg, and in female students 3.7, as compared to the data recorded at the level of 170 meters.

4. The average level of systolic blood volume of females under the conditions of middle mountains

---

**Figure 3. Cardiovascular system of female students in conditions of hypoxia**
has increased by 1.6 ml, and peers by 2.5 (P <0.05), as compared to the data reported for the plain.

5. The range of the MBV and EF indicators between groups of young males has demonstrated changes from 4.4 and 17.4 liters to 4.5 and 18.4, and peers from 4.7 and 18.2 to 4.8 and 18.7, respectively.

6. The value of TPVR at an altitude of 600 meters is lower by 52 dyn·s·cm⁻⁵ for males, and in the middle mountains by 24 for females.

7. The drop in CEC for the group of female students living in the middle mountains has been recorded to be 194 and 124 for peers, respectively, as compared to the data obtained under the plain conditions.

Statement on ethical issues

Research involving people and/or animals is in full compliance with current national and international ethical standards.

Conflict of interest

None declared.

Author contributions

The authors read the ICMJE criteria for authorship and approved the final manuscript.

References


10. Latyshev VS. Comparative characteristics of the parameters of resistance to hypoxia in boys and girls from different regions. XXII All-Russian Scientific and Practical Conference of Nizhnevartovsk State University. April 06–07, 2020 Publisher: Nizhnevartovsk State University, 2020. 90-93. [in Russian]


17. Shaov MT, Pshikova OV, Kurdanov KhA. Neuroimprinting – technologies for controlling the physiological functions of the body and human health


Features of life activity and the incidence rate of anxiety and depressive disorders among medical students studying remotely during the epidemic of a new coronavirus infection (Covid 19)

Aksana M. Kardangusheva1, Diana Z. Kaskulova1, Maryam H. Kurdanova1, Antonina F. Budnik1, Olga V. Voronova1, Zukhra A. Gelyakhova1

1Kabardino-Balkarian State University named after H.M. Berbekov, 360004, Russia, Kabardino-Balkarian Republic, Nalchik, Chernyshevsky St., 173
2Rostov State Medical University, Russian Federation, 344000, Rostov-on-Don, Nahichevansky av., 29

*Corresponding author: kardangush@mail.ru
+7(964)0311050

Abstract

**Aims.** The aim is to study the features of life and the incidence rate of anxiety and depressive disorders among medical faculty students studying remotely during the epidemic of a new coronavirus infection (Covid 19) at the Kabardino-Balkarian State University named after H.M. Berbekov.

**Materials and methods.** We examined 335 students (34% males, 66% females) at the Faculty of Medicine in November-December 2020. The average age of the students examined was 20.3±2.3 years. The study included an anonymous mail survey to examine educational conditions, lifestyle, levels of anxiety and depression.

**Results.** 43.8% of the students had insufficient sleep duration, 58.5% had low physical activity, in 24.8% of them we recorded high levels of situational anxiety and in 82.1% of them personal anxiety, in 7.1% of the students we recorded clinically expressed anxiety and 1.8% had clinically expressed depression. During the transition to remote learning, students increased the time use to prepare for classes (51.2%) and the duration of sleep (61.5%), while their physical activity decreased (76.5%). Statistically significant linear correlations were identified between the level of anxiety and depression and the duration of sleep, low physical activity, preparation time for classes, academic performance, and academic debt.

**Conclusions.** The results obtained by us can be used to optimize the educational process, as well as to preserve the mental health of students during the transition to distance learning.

**Keywords**

Students, Distance learning, Lifestyle, New coronavirus infection (Covid 19), Situational anxiety, Personal anxiety, Depression

Imprint


Due to the spread of the new coronavirus infection (Covid 19), many higher education institutions around the world have decided to manage distance learning. According to the International Association of Universities, some colleges and universities continued full-time education, some switched to mixed or hybrid education, while other used part- or full-time online education only [1]. The transition to distance learning made it possible to ensure the continuity of the educational process. Along with the pedagogical aspects of education, maintaining the health of students is of great importance. The organization of education, which is unusual for most students, the alarming epidemic situation, and the restrictions on life activity associated with the pandemic could have an impact on the health of students [1,2].

Currently, the results of scientific studies on the impact of the pandemic on university students have been published. The largest number of publications is devoted to studies of students’ adaptation to online learning and the impact of Covid-19 on students’ mental health [2-8]. There are fewer studies on the impact of the pandemic on academic, social aspects, health, lifestyle and behavioral elements of students [9, 10].

In our study, we focused more on the specifics of the learning environment and lifestyle of distance learning medical students during the Covid-19 pan-
emic. In our opinion, this problem is of particular relevance for students of medical universities due to their exposure to high psychological, emotional, physical and intellectual stress in the learning process [3].

The aim hereof is to study the characteristics of life and the incidence rate of anxiety and depressive disorders among students of the Medical Faculty of the Kabardino-Balkarian State University named after H.M. Berbekov (KBSU), who were studying remotely during the epidemic of a new coronavirus infection (Covid 19).

Material and methods

The study was conducted at the Faculty of Medicine at KBSU in November-December 2020. During the study at KBSU, the students were taught remotely. Distance learning was organized mainly in the format of digital information system Open University at KBSU under open.kbsu.ru. Taking into account the capabilities of students (the presence and type of a gadget, the availability of Internet access and the stability of the Internet connection), Zoom, WhatsApp, etc. were also used. Remote practical, seminar and lecture online classes were held, as well as the exchange of educational information (tasks, abstracts, essays, etc.) using various digital devices. Personal contact between lecturers and students was excluded.

We interviewed 335 students (34% (n=114) males, 66% (n=221) females) of 3-4 courses, Medicine specialty. The average age of the surveyed students was 20.3±2.3 years. The majority of students were not married (85.6%). Only 14.4% of the students, of which 22.5% of the males and 77.5% of the females were married (p<0.0001). Among the students we interviewed, 4.7% (6.8% of males and 3.6% of females) were employed. In the structure of positions of employed students, the positions of paramedic (12.5%), nurse (37.5%) and orderly (18.8%) prevailed.

We studied an anonymous mail survey to examine educational conditions, lifestyle, levels of anxiety and depression. The questionnaire contained questions that allowed assessing marital status, education, information about employment, level of physical activity, academic performance, academic debt, time used to prepare for classes, duration of classes, sleep duration, as well as respondents’ assessment of changes in learning conditions, lifestyle when switching to distance learning. To determine the level of situational anxiety (SA) and personal anxiety (PA), testing was carried out according to the Spielberger-Khanin method [11]. When assessing levels of anxiety, a score under 30 was assessed as low anxiety; from 31 to 45 – moderate anxiety and from 46 or over – high anxiety [11]. Anxiety and depression levels were measured using the HADS Anxiety and Depression Scale developed by A.S. Zig mond and R.P. Snaith in 1983 [12]. When assessing the levels of anxiety and depression, a score under 8 is regarded as the absence of significantly expressed symptoms of anxiety and depression, 8-11 as subclinically expressed anxiety/depression, 12 and over as clinically expressed anxiety/depression [12]. Physical activity was assessed according to the Global Questionnaire on Physical Activity recommended by the World Health Organization. The group of persons with low physical activity (LPA) included students whose moderate-intensity physical activity was less than 150 minutes per week, or high-intensity physical activity less than 75 minutes per week [13].

Statistical analysis of the data was carried out using STATISTICA 6.0 software (StatSoft Inc, USA). The results are presented as the mean and its standard deviation for continuous variables and as a percentage for categorical variables. Hypotheses about a possible relationship between individual quantitative variables were refined by the method of paired correlation analysis. The results of the correlation analysis were interpreted on the basis of the Spearman criterion, while determining the rank correlation coefficient r. The values of p<0.05 were taken as the critical level of significance when testing statistical hypotheses.

Results

The average sleep duration of students is 7.5±1.5 hours. As shown in Figure 1, 43.8% of students sleep less than 8 hours a day (44.8% of the females and 41.9% of the males).

It is noteworthy that the duration of sleep has changed in 70.3% of students (70.9% of the males and 70% of the females). Moreover, in 61.5% of the students (63.2% of males and 60.5% of females) the duration of sleep increased. The distribution of students according to the time of increasing the duration of sleep is shown in Figure 2 herein.

In 48.3% of the students (52% of the males and 46.6% of the females) the sleep duration increased by 2 hours and in 23% (26% of the males and 21.5% of the females) by 3 hours, respectively. Gender differences were statistically significant among those students which demonstrated an increase in the duration
of sleep by 1 hour (1% of the males and 10.4% of the females, p<0.01) and by 4 hours (20% of the males and 1.2% of the females, p<0.0001).

Along with this, 12.6% of the students (7.7% of the males and 9.4% of the females) reported a decrease in sleep duration. The distribution of students according to the time of the sleep duration decrease is shown in Figure 3 herein. It is noteworthy that in most students the duration of sleep decreased by 2 hours (77.7% of the males and 42.9% of the females, p<0.0001) and 3 hours (22.2% of the males and 23.1% the females).

More than half of the students we surveyed (58.5%) were found to have LPA. Moreover, LPA was more common among females than among males (64.8% versus 46.6%, p<0.01). It is noteworthy that 87.2% of the students (87.9% of the males and 86.8% of the females) indicated changes in their physical activity during distance learning. Among the students who indicated the nature of changes in physical activity (33.6%), 76.5% of the students reported a decrease in physical activity (56% of the males and 83.6% of the females, p<0.0001), and an increase was reported in 23.5% of the students (44% males and 16.4% females, p<0.0001).

Teaching hours spent on online learning varied from 1 hour to 8 hours per day, averaging 5.6 ± 3.3 hours. Moreover, 81.5% of students spent 5-6 hours per day on online learning, 10 % - 4 hours, 5.9% - 1-2 hours, 2.6% - 7-8 hours. The time for preparing students for classes varied from 1 hour to 16 hours (see Figure 4 herein), averaging 6.0± 1.9 hours. More than a third of the students we surveyed spent 6 hours preparing for classes.

The time of preparation for classes during the transition to distance learning has changed for 57.7% of the students (59% of the males and 57% of the females). Moreover, 51.2% of the students (53.9% of the males and 49.8% of the females) increased the time of preparation for classes, and 6.5% of the students (5.1% of the males and 7.2% of the females) decreased it. In the distribution of students according to the change in the time of preparation for classes, gender differences were identified (see Figure 5,6 herein). In the males, the preparation time for classes increased by 1 hour and 8 hours, more often than it was the case with the females (p<0.01); in the females we recorded an increase by 2 hours and 4 hours more often than the respective data in the males (p<0.05; p< 0.01).
Figure 3. Distribution of students according to the time of decrease in sleep duration (%)

Figure 4. Distribution of students by time of preparation for classes (%)

Figure 5. Distribution of students by increase in preparation time for classes (%)

Figure 6. Distribution of students by reduction of preparation time for classes (%)

Issue 21. February 2022 | Cardiometry | 133
the males, the time of preparation for classes also decreased by 4 hours more often than among the females (p<0.01).

17.1% of the students had academic debts (13.7% of the males and 18.8% of the females). The distribution of the students by the number of academic debts is shown in Figure 7 herein. The females more often than the males had 1 debt (p<0.0001), and the males more often than the females had 2 and 4 debts (p<0.0001, p<0.01). The presence of 3 and 5 debts was indicated only by the females, and 6 debts only by the males.

Most of the students we interviewed (63.8%) indicated good academic performance (see Figure 8 herein).

Among the examined students, moderate levels of SA were determined (43.6±4.8 points) and high levels of PA (49.1±5.2 points, Table 1). Low levels of anxiety were detected only in three students, of which in two cases a low SA was determined, and in one case a low PA was recorded. Most of the students we interviewed had an average level of SA (74.6%) and a high level of PA (82.1%). The prevalence of high SA among all students was 24.8%. The gender differences in SA and PA levels did not reach the level of statistical significance.

The prevalence of anxiety and depression among students according to the HADS scale is presented in

![Figure 7. Distribution of students by the number of academic debts (%)](image_url)

![Figure 8. Distribution of students by academic performance (%)](image_url)

<table>
<thead>
<tr>
<th>Levels</th>
<th>Situational anxiety</th>
<th>Personal anxiety</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males</td>
<td>Females</td>
</tr>
<tr>
<td>Average values, points (M±δ)</td>
<td>43.7±5.0</td>
<td>43.6±4.7</td>
</tr>
<tr>
<td>Low, n (%)</td>
<td>0</td>
<td>2 (0.9)</td>
</tr>
<tr>
<td>Moderate, n (%)</td>
<td>83 (72.8)</td>
<td>167 (75.6)</td>
</tr>
<tr>
<td>High, n (%)</td>
<td>31 (27.2)</td>
<td>52 (23.5)</td>
</tr>
</tbody>
</table>
Table 2 herein. Symptoms of clinically expressed depression were recorded only in 1.8% of the students, and symptoms of clinically expressed anxiety in 11.8% of them. Gender differences did not reach the level of statistical significance.

To assess an impact of high anxiety, levels of anxiety and depression on other indicators studied by us, we conducted a correlation analysis. As shown in Table 3, a strong direct correlation was obtained between the level of PA and a decrease in the time of preparation for classes during the transition to distance learning; the severity of anxiety and an increase in the preparation time for classes when switching to distance learning. Moderate and inverse correlations were identified between the severity of anxiety and LPA; between the level of PA and the duration of sleep and a decrease in physical activity during the transition to distance learning. Medium strength and direct correlations have been found: between the severity of depression and a decrease in the time to prepare for classes and a decrease in physical activity during the transition to distance learning; between PA and the amount of debts. Weak strength and inverse correlations have been detected between the severity of anxiety and the duration of sleep, between the severity of depression and the duration of sleep and LPA; between PA and LPA. Weak strength and direct correlations have been revealed: between the severity of anxiety and the presence of academic debt; between the severity of depression and the time to prepare for classes, academic performance, the presence of academic debt, an increase in sleep duration and time to prepare for classes when switching to distance learning. In relation to SA, correlations of weak strength and direct correlations with academic performance, academic debt and LPA were obtained, in relation to PA – with the time of preparation for classes and the presence of academic debt.

Table 3
Correlations between the levels of anxiety and depression with some of the studied indicators (p<0.05)

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Anxiety</th>
<th>Depression</th>
<th>Personal anxiety</th>
<th>Situational anxiety</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sleep duration</td>
<td>-0.24</td>
<td>-0.24</td>
<td>-0.35</td>
<td></td>
</tr>
<tr>
<td>Increase in sleep duration when switching to distance learning (hours)</td>
<td>0.25</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preparation time for classes (hours)</td>
<td></td>
<td>0.11</td>
<td>0.16</td>
<td></td>
</tr>
<tr>
<td>Increase in preparation time for classes when switching to distance learning (hours)</td>
<td>0.89</td>
<td>0.16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Decrease in the preparation time for classes when switching to distance learning (hours)</td>
<td>0.45</td>
<td>0.96</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Academic performance</td>
<td></td>
<td>0.18</td>
<td>0.22</td>
<td></td>
</tr>
<tr>
<td>Academic debts</td>
<td>0.14</td>
<td>0.16</td>
<td>0.14</td>
<td></td>
</tr>
<tr>
<td>Number of academic debts</td>
<td></td>
<td></td>
<td>0.35</td>
<td></td>
</tr>
<tr>
<td>Low physical activity</td>
<td>-0.33</td>
<td>-0.22</td>
<td>-0.23</td>
<td>0.15</td>
</tr>
<tr>
<td>Increase in physical activity during the transition to distance learning (hours)</td>
<td></td>
<td></td>
<td>0.41</td>
<td></td>
</tr>
<tr>
<td>Decrease in physical activity during the transition to distance learning (hours)</td>
<td></td>
<td></td>
<td>-0.33</td>
<td></td>
</tr>
</tbody>
</table>

Discussion
The pandemic has produced a great impact on the activities of higher education institutions and the higher education system: millions of students around the world study remotely [1], and it is not known when higher education institutions will return to the traditional format of education, and whether they will return. In this regard, it is necessary to understand the patterns of changes in the life of students arising in connection with the pandemic and their impact on mental health. Important components of the life of students include adequate sleep duration, optimal physical activity, a healthy psychological atmosphere in their environment and the effective organization of the educational process. When studying the characteristics of the life of the students at the KBSU Fac-
ulty of Medicine, who were studying remotely during the Covid-19 pandemic, we found that almost every second student still has insufficient sleep, despite its increase with the transition to distance learning. The high prevalence of LPA and its increase with the transition to distance learning characterized the students we surveyed. Moreover, the most unfavorable situation in terms of LPA in the group of the females, among which both the prevalence of LPA and an increase in the latter during distance learning, is higher than among males. The high prevalence of LPA and its increase with the transition to distance learning have been established by other researchers [2,4,5,8]. J.F. Huckins et al (2020) notes that American college students have reduced their physical activity and are visiting fewer places, while reporting increased symptoms of anxiety and depression [5].

With regard to teaching hours spent on online learning, it can be concluded that in most cases, students were covered by online classes in accordance with the schedule. This is a teacher-controlled part of the learning process. Therefore, the study load in hours, according to the results of the survey of the students, does not differ from the study load in the curricula of the corresponding courses. As for the preparation time for students’ classes, its variability and increase in the transition to a distance learning format for half of the students we surveyed can be associated both with the low adaptation of students to online learning and the need to reconsider the workload on the student. This can also be indicated by the presence of academic debt recorded for every sixth student. Despite the increase in preparation time for classes as a result of the transition to distance learning, the progress of most students is good that may indicate their high motivation. Summarizing the above, we can conclude that the university staff has done a good job organizing distance learning, and the recommendation to adapt students to online learning and review the workload on the student, in our opinion, can improve the quality of distance learning.

The Covid-19 pandemic, which has disrupted the daily lives of people around the world, can certainly put people at greater risk of mental health problems. Researchers consider high anxiety and depression as consequences of a viral pandemic [14]. When studying the anxiety level of the KBSU students, we have found a high prevalence of PA. It is known that PA is associated with genetically determined properties of the functioning human brain, which cause a constant-ly elevated level of emotional arousal and, along with increased SA caused by various stress factors, contributes to the development of distress and psychosomatic pathologies [11].

In the context of the Covid-19 pandemic, symptoms of mental disorders are of growing concern around the world [4-8]. According to the Healthy Minds Study [15], more than 50% of American college students are concerned about becoming infected by Covid-19, and about 90% are concerned about their personal safety. Students also have a lower level of psychological well-being than before the pandemic [15]. A.H. Khan et al (2020) identify stress, anxiety and depression as common symptoms among Bangladeshi college students and fear of contracting Covid-19 as the main cause of these symptoms [7]. Studies conducted in Chinese colleges show that students are concerned about delays in their studies, negative economic consequences, routines and daily activities [6].

Our correlation analysis identified some statistically significant linear relationships of various strengths between the severity of anxiety and depression and the level of anxiety, on the one hand, and some lifestyle factors (sleep duration and its changes, low physical activity, changes in physical activity) and the educational process (time to prepare for classes and its change, academic performance, academic debt) on the other hand. Although the correlation does not directly indicate a causal relationship, it can be assumed that the factors we have identified can influence both the level of anxiety and the severity of anxiety and depression. These factors are quite flexible and easily manageable. Rational modulation and control over them can neutralize high anxiety, as well as manifestations of anxiety and depression. There is no doubt that recommendations on the organization of sports or physical exercises and the rational distribution of preparation time for classes for students in distance learning can be useful.

Conclusions

Thus, we have studied the characteristics of life activity and the incidence rate of anxiety and depressive disorders among the students at the KBSU Faculty of Medicine, who were studying remotely during the Covid 19 pandemic, as well as the relationship between the state of mental health of students and the parameters of their life included in the study. We have found that during the transition to distance learning in the life of the KBSU students, there are unfavorable
changes for their health in the form of a decrease in physical activity, an increase in the time for preparing for classes, and favorable changes in the form of an increase in the duration of sleep. We have shown that the students of the Medical Faculty at KBSU have a very high level of anxiety and developing anxiety-depressive disorders. Some statistically significant linear correlations have been detected between the level of anxiety and depression on the one hand and the duration of sleep, changes in sleep duration, LPA, changes in physical activity, time to prepare for classes and its changes, academic performance and the presence of academic debt, on the other hand. Knowledge of the factors contributing to the emergence of anxiety and depressive disorders in students will be useful for improving the educational process at the Faculty of Medicine at KBSU. The results obtained by us can be used to optimize the academic process, as well as to preserve the mental health of students during the transition to distance learning.

Statement on ethical issues
Research involving people and/or animals is in full compliance with current national and international ethical standards.

Conflict of interest
None declared.

Author contributions
The authors read the ICMJE criteria for authorship and approved the final manuscript.

References