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COMPULSORY LICENSING IN PHARMACEUTICAL INDUSTRY: CURRENT STATE OF AFFAIRS AND PROSPECTS

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The problem of compulsory licensing (CL) in the pharmaceutical industry is being discussed worldwide. The aim of this paper was to analyze the effects of using CL for pharmaceutical drugs (PD) as part of competitive policies aimed at safeguarding the life and health of the population. Using PEST-analysis, we identify the main political, economic, social and technological problems associated with using CL in the pharmaceutical industry. We demonstrate the potential of CL as a tool for countering the threats to public health caused by the abuse of market dominance by pharmaceutical patent holders. At present, both developers of pharmaceutical innovations (patent-holders) and other entities involved in drug circulation are protected by law. There is ongoing debate about the efficacy of CL as a tool ensuring the implementation of competitive policies aimed at safeguarding the rights to life and health. However, in Russia CL is applied only under exceptional circumstances. An economic balance should be sought between the incentives for innovation, long-term profits from selling PDs and PD accessibility.

Keywords: compulsory licensing, competition in pharmaceutical industry, drugs, PEST-analysis.

Author contribution: Gaydin TY analyzed the literature, conducted the study and wrote the draft of the manuscript; Rozhnova SA planned and conducted the study, analyzed the literature and wrote the draft of the manuscript

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ПРИНУДИТЕЛЬНОЕ ЛИЦЕНЗИРОВАНИЕ В ФАРМАЦИИ: ТЕКУЩЕЕ РЕГУЛИРОВАНИЕ И ПЕРСПЕКТИВЫ

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Проблема применения принудительного лицензирования (ПЛ) актуальна для фармацевтических рынков во многих странах. Целью работы было проанализировать последствия применения ПЛ в системе обращения лекарственных средств (ЛС) для обеспечения охраны жизни и здоровья граждан в рамках конкурентной политики на рынке ЛС. С помощью PEST-анализа определены экономические, социальные, технологические и политические проблемы, связанные с ПЛ в фармации. Показаны возможности применения ПЛ как инструмента конкурентной политики для регулирования случаев злоупотреблений фармацевтическими компаниями-патентообладателями доминирующим положением на российском рынке ЛС, что приводит к снижению благосостояния потребителей и угрожает жизни и здоровью граждан. На сегодняшний день законодательством защищены как патентообладатели в сфере фармацевтической разработки, так и субъекты обращения ЛС. Продолжается дискуссия об эффективности возможного ПЛ как инструмента конкурентной политики для охраны жизни и здоровья граждан, но на сегодняшний день ПЛ в России применяют в исключительных случаях. Необходим поиск экономического баланса между стимулами к инновационной активности компаний, окупающих вложения в исследования и разработки, долгосрочным доходом с продажи ЛС и доступностью ЛС.

Ключевые слова: принудительное лицензирование, конкурентная политика в фармации, лекарственные средства, PEST-анализ

Вклад авторов: Т. Ю. Гайдин — работа с материалами, проведение исследования, подготовка черновика рукописи; С. А. Рожнова — планирование и проведение исследования, анализ литературы, подготовка черновика рукописи.

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Weak regulatory policies on compulsory licensing (CL) are a setback to countering monopolies on the Russian pharmaceutical market. Recognizing CL as a legal tool for protecting the fundamental right to life and health might be a promising strategy. The world is faced with economic, political and social challenges that are escalating tensions in the pharmaceutical market and affecting the availability of pharmaceutical drugs (PDs). The mechanisms used to relieve these tensions at the national level include patent legislation and adequate drug supply to the population. At the international level, CL can be applied. In Russia, the Government has the right to authorize the use of a patented invention without the patent holder's consent in the interest of national defense and public safety. The patent holder must be promptly notified of the decision and is entitled to adequate remuneration [1]. The procedure is regulated by the Civil Code of the Russian Federation.

Innovative PDs are used to prevent, diagnose and manage diseases that could not be treated or cured in the past and to rehabilitate the affected patients [2]. Development of an innovative PD is a long, difficult, knowledge-intensive and costly process associated with a plethora of risks threatening to suspend or terminate the project [3]. A PD patent offers its proprietor the chance to recoup the costs of drug development, registration and marketing. Once a PD has been approved and registered, the patent holder has the right to decide on how and where the invention can be exploited. Due to the abuse of a dominant position by pharmaceutical companies, public access to some essential original drugs is limited.

Intellectual property law is the primary mechanism for recouping investments in drug development. Throughout the exclusivity period, a holder of a composition-of-matter, production technology or method-of-use patent remains the only

Table 1. Examples of using CL in the pharmaceutical sector

International nonproprietary name of a PD or an active substance	Manufacturer		PL issuing country and year	Number of legal proceedings	Grounds
	Original drug	Generic			
Lenalidomide	Celgene International Holdings Corporation	Nativa, OOO	Russia, 2018	1	Patent dependency
Lopinavir Ritonavir	Abbot	State-partnered companies	Brazil, 2008	1	Abuse of market dominance
Efavirenz	Merck Sharp & Dohme	State-partnered companies	Brazil, 2007	1	
Sorafenib	Bayer	Natco Pharma Ltd.	India, 2012	1	
Lovastatin	Private company	State-partnered companies	Canada, 1980	1	

representative of the patented product in the pharmaceutical marker. This allows the patent holder to address economic issues associated with product development.

However, patent law sometimes clashes with national security, the right to health, public welfare and technical progress. CL, which restricts the rights of a patent holder, is one of the mechanisms devised by the state to regulate such disagreements.

First and foremost, patent rights can be restricted in the best interest of national defense and security. The cases of conducting a research study, preparing a drug at a pharmacy for an individual patient, using a patented innovation in the event of emergency, etc. are not considered an infringement of the exclusive rights of a patent proprietor [1].

A compulsory license cannot be issued on account of overpricing, allowing pharmaceutical companies to abuse their dominant position in the market. Up to this day, there are no effective regulatory policies to protect against such abusive practices. A draft bill, which is currently under consideration in the State Duma, seeks to broaden the mandate of the Government so as to limit the rights of patent holders in the best interest of national defense and to uphold the rights to life and health [4].

In Russia, patent laws protecting the rights of a PD patent holder conform to international standards. So far, CL has not been used to resolve disagreements between pharmaceutical companies. However, in 2018 a compulsory license was issued by the court of law to allow a Russian-based pharmaceutical manufacturer Nativa to produce an analogue of a drug patented by the Celgene International Holdings Corporation; the situation sparked a lot of debate in the pharmaceutical sector [5].

Aspects and prospects of CL

We collected and organized data on the use of CL worldwide [6–10]; the results are presented in Table 1.

Table 1 illustrates that at the international level, legal grounds for issuing a compulsory license for a PD are limited to PD exclusiveness.

In Russia, the first compulsory license for a patented medicinal product was issued in 2018. Initially, the original drug was patented by Celgene International Holdings Corporation. A suit against Celgene was filed by the Russian company Nativa on grounds of patent dependency and following the refusal of the patent holder to license the drug to Nativa [5]. In other words, the compulsory license was issued because one patent was dependent on the other [6] but not because competition policy was being pursued to safeguard the life and health of the population. A significant reduction in the selling price was the main positive outcome of the court's decision [11].

Table 1 features cases of CL due to the abuse of market dominance by drug manufacturers [7–10]. In its current state, Russian legislation does not contain any provisions protecting against the abuse of a dominant position by a pharmaceutical company.

For this study, we compared wholesale prices for the original patented drug Revlimid (lenalidomide) and its generic using archived data from the State Registry of Maximum Wholesale Prices dated 08.06.2018 (Table 2).

CL as factor for safeguarding the life and health

To assess the feasibility of using CL as a tool for safeguarding the life and health of the population, we performed the PEST analysis of external political, economic, social, and technological factors that might influence the decision to issue a compulsory license for the generic drug Lenalidomide-Nativ. The factors were ranked by the force of impact in the descending order. For each factor, the force of impact was evaluated on a scale from 1 to 3 points, where 1 point represented mild impact, 2 points represented moderate impact and 3 points represented strong impact. For each external factor, the probability of change was estimated on a 5-point scale, where 1 point represented the minimal likelihood, and 5 points represented the maximum probability. Weight-corrected scores were obtained by multiplying the force of impact determined for the studied factor by the probability of change and dividing the resultant value by 34, i.e. the total impact of the factor [12, 13].

Weight-corrected scores represent the real significance of the studied factors in determining the decision to issue the compulsory license for the drug and launch its domestic

Table 2. Comparison of maximum wholesale prices for the original drug lenalidomide and its generic (from https://grls.rosminzdrav.ru/)

Dosage forms, strengths and package sizes	Maximum price, roubles		Difference in maximum price	Reduction in maximum price, %
	Manufactured by Celgene Corporation under trade name Revlimid	Manufactured by Nativa under trade name Lenalidomide-Nativ		
Seven 25 mg capsules	453 069.75	211 584.80	241 284.95	53%
Seven 10 mg capsules	422 000.00	122 787.00	299 213.00	71%
Seven 5 mg capsules	422 000.00	73 101.00	348 899.00	83%
Seven 15 mg capsules	443 100.00	172 389.00	270 711.00	61%

Table 3. The analysis of external political, economic, social, and technological factors (PEST analysis) that might influence the decision to issue a compulsory license for the generic lenalidomide drug

Factor		Force of impact	Probability of change	Weight-corrected score
Political factors				
1	CL-related changes in the degree of intellectual property protection	3	5	0.44
2	Possible use of CL for safeguarding the life and health of the population	3	3	0.26
3	Regulation of the pharmaceutical industry by the state: the registry of essential drugs, which already includes lenalidomide-based drugs	2	1	0.06
4	A new method for registering an essential drug price	2	3	0.18
5	Amendments to drug procurement legislation (priority is given to Russian-based companies; local content policies)	1	3	0.09
6	Commitment of the state to a plethora of social policies articulated in the Constitution and other statutory laws regulating drug provision (drug procurement activities of the state have not decreased substantially, as compared to the activities on the general pharmaceutical market, in terms of value and considering the inflation rate)	1	1	0.03
Social factors				
1	The need for accessible lenalidomide	3	2	0.18
2	The significance of a welfare state concept	2	1	0.06
3	Patients' awareness about the original drug and its generic affects the demand (including the demand for lenalidomide)	2	4	0.24
4	Demographics: population ageing	1	1	0.03
5	The need for better quality of life and improved performance in the workplace in the face of increasing retirement age	1	2	0.06
Economic factors				
1	Cutting state expenditures on PD procurement under targeted programs for drug provision	3	4	0.35
2	Falling incomes of the population	2	3	0.18
3	The impact of national currency dynamics on the price of PD or its components	2	2	0.12
Technological factors				
1	Insufficient investments in R&D in the pharmaceutical industry impeding the launch of innovative PD	3	3	0.26
2	Insignificant export volumes (for both original PD and generics)	2	4	0.24
3	Limited access to state-of-the-art technologies for lenalomide-based PD production	1	3	0.09
Total:	34	45		

Note: R&D — research and development.

production. The higher the real significance of the factor shown in Table 3, the more effort is needed to reduce its negative impact. The results of the PEST-analysis are shown in Fig.

The analysis revealed that weakened intellectual property protection due to CL is the crucial political factor affecting the decision to launch the production of a generic; in our case this might drive the manufacturer of the original drug out of the Russian market. The registry of essential medicines is a mechanism of market regulation by the state: it allows the state to set a fixed price for a PD. Once a drug is excluded from the registry, the doors for competitive pricing will be opened because the manufacturer will no longer have to register the price for the product with the state. The most influential economic factor involves the cutting of state expenditures on drug procurement, which may give a competitive advantage to a generic. The most important social factor is patients' awareness about the original drug and its generic, because the negative opinion about the generic drug affects the demand.

Thus, CL for medicinal drugs aimed at protecting the life and health of the population may produce undesirable economic and other effects on the pharmaceutical market.

CL limits the rights of a patent-holder in cases when mass production of a drug is needed. In Russia, a compulsory license can be legally issued by the court of law following a third party's claim. The patent will be licensed to the third party if the court decides that there are sufficient grounds for CL. The terms and conditions for CL are determined by the court.

In Russia, there are only 2 legal grounds for issuing a compulsory license. The first is the non-use of an innovation (a medicinal product in our case), a patented production prototype or a useful model over a certain time period. If the innovation is not produced in sufficient quantities to satisfy the market demand and the patent holder has refused to license the innovation to the third party that possesses the capacities to launch mass production of the innovation, the third party has the right to file a legal claim for the compulsory license. If the patent holder fails to prove that they were facing insurmountable obstacles preventing them from signing the agreement, the court is likely to issue a compulsory license to the third party.

The second legal ground for issuing a compulsory license is patent dependency. If an innovation cannot be manufactured without exploiting another patented innovation that bears no relation to the holder of the dependent patent, the latter has the right to seek a compulsory license for the original patent in court. The terms and conditions of patent transfer are specified by the original patent holder and determined by

Fig. The results of the analysis of external political, economic, social and technological factors that impact the decision to launch production of lenalidomide-based generic drugs

the court. The obtained license cannot be transferred to other parties expect for cases of dependent patent transfer [1]. New regulatory mechanisms are being discussed to sort out such disagreements. A patent holder faces a number of additional risks of losing control over the invention. Similarly, the state faces a number of economic and non-economic problems (political, legislative, ethical) when trying to ensure that the demand for essential drugs is satisfied.

The price for an essential drug is established based on of the wholesale price, wholesale and retail markups [14]. Limits to wholesale and retail markups are set by the state. The wholesale price set by the manufacturer is the sum of production costs and profits. According to the Russian law on drug circulation, the maximum wholesale price set by a manufacturer for the drug included in the registry of essential medicinal products must be registered with the state. If the costs of production are low and the wholesale price is too high, public access to the drug will be limited. The state should seek to create a fair and economically justified balance of interests between the manufacturer and the consumer when fair competition and public wellbeing are at risk and the conflict of interests cannot be solved without state intervention. Damage to public welfare is not the sufficient reason for the manufacturer of the original drug (the monopolist) to lower the wholesale price; on the contrary, the manufacturer will raise the price, pointing to the substantial R&D investments. The price might go down due to fair competition, but in order to produce a competitive generic, the rivaling company needs the manufacturing technology normally protected by the patent. So, in the event of market dominance abuse, CL might be viewed as a safeguard of the population's life and health.

The relationship between competitive policies and innovation activities in different sectors of the economy is an attractive object of research [15–17]. Some authors describe CL as a mechanism for the implementation of competitive policies associated with the innovation activities of companies [16–18]. Some researchers [18–21] have proposed economic models suggesting that CL can significantly improve consumer welfare in some cases. Using an original model, researchers have demonstrated that CL effects on consumer welfare depend on the level of competition in the field; if the market is not competitive, CL will improve consumer welfare [18]. There are different ways of PD commercialization. Sometimes, intellectual property licensing can be applied: the right to launch mass production of the product is granted to another company on certain terms but the developer of the product retains the patent for the manufacturing technology. Often, the developer sells the manufacturing technology to another company. The most difficult and costly way is to launch independent production of the PD using the original manufacturing technology [22]. In such cases, CL should be applied with caution so as not to do irrevocable damage to the parties involved and not to violate the law.

The results of PEST analysis suggest that safeguarding the life and health of the population is the sufficient ground for issuing a compulsory license; it also improves public welfare in the short term.

There is a lot of controversy about using CL as a mechanism to regulate competition [23]. When viewed as a factor ensuring the right to life and health, CL is used as a component of competitive policies. It is reported that CL was effective in countering the abuse of a dominant position in the domestic market during the COVID-19 pandemic [24]; however, our findings suggest that the results of applying CL might be controversial.

Conclusion

At present, the right to life and health cannot be appealed to as the legal ground to issue a compulsory license for medicinal products in Russia. Although CL poses significant risks for market competition, its regulatory potential can be estimated as powerful.

As a factor safeguarding the life and health of the population, CL can significantly improve consumer welfare as it makes essential drugs more accessible to consumers. CL will be instrumental in countering the abuse of market dominance by pharmaceutical companies.

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LEVELS OF MIR-374 INCREASE IN BEWO B30 CELLS EXPOSED TO HYPOXIA

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In humans, trophoblast hypoxia during placental development can be a cause of serious pregnancy complications, such as preeclampsia and fetal growth restriction. The pathogenesis of these conditions is not fully clear and may be associated with changed expression of some genes and regulatory molecules, including miRNA, in trophoblast cells. The aim of this study was to analyze miRNA profiles and measure the expression of their target genes in a model of trophoblast hypoxia. Human choriocarcinoma BeWo b30 cells were used as a trophoblast model. Hypoxia was induced by cobalt chloride ($CoCl_2$) and an oxyquinoline derivative. MRNA and miRNA expression profiles were evaluated by means of next generation sequencing (NGS); the expression of individual genes was analyzed by PCR. We studied the secondary structure of mRNAs of target genes for those miRNAs whose expression had changed significantly and analyzed potential competition between these miRNAs for the binding site. The observed changes in the expression of the key genes involved in the response to hypoxia confirmed the feasibility of using $CoCl_2$ and the oxyquinoline derivative as hypoxia inducers. The analysis revealed an increase in miR-374 levels following the activation of the hypoxia pathway in our trophoblast model. The changes were accompanied by a reduction in *FOXM1* mRNA expression; this mRNA is a target for hsa-miR-374a-5p and hsa-miR-374b-5p, which can compete with hsa-miR-21-5p for the binding sites on *FOXM1* mRNA. The involvement of *FOXM1* in the regulation of the invasive cell potential suggests the role of miR-374 and *FOXM1* in the pathogenesis of disrupted trophoblast invasion during placental development as predisposing for fetal growth restriction and preeclampsia.

Keywords: placenta, choriocarcinoma, BeWo, hypoxia, cobalt, oxyquinoline, *FOXM1*, microRNA, miR-374a-5p, miR-374b-5p

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Author contribution: Knyazev EN analyzed the literature, planned the study, collected and interpreted the obtained data, and wrote the manuscript; Paul SYu analyzed the literature and the obtained data and wrote the manuscript.

Compliance with ethical standards: the study complied with the Declaration of Helsinki.

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УРОВЕНЬ микроРНК MIR-374 ПОВЫШАЕТСЯ В КЛЕТКАХ BEWO B30 ПРИ ГИПОКСИИ

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Воздействие гипоксии на клетки трофобласта в процессе формирования плаценты человека может приводить к развитию ряда патологий беременности, таких как преэклампсия и задержка роста плода. Патогенез данных состояний не до конца раскрыт и может быть связан с изменением экспрессии в трофобласте ряда генов и регуляторных молекул, включая микроРНК. Целью данного исследования было изучить профили микроРНК и экспрессии соответствующих генов-мишеней в модели гипоксии трофобласта. Моделирование трофобласта проводили с использованием клеточной линии хориокарциномы человека BeWo b30. В качестве индукторов гипоксического ответа использовали хлорид кобальта ($CoCl_2$) и производное оксихинолина. Анализировали уровень мРНК и микроРНК с помощью секвенирования следующего поколения (NGS) с подтверждением экспрессии отдельных генов ПЦР. Анализировали вторичную структуру мРНК гена-мишени значимо изменившихся микроРНК и возможную конкуренцию за места связывания. Изменение экспрессии ключевых генов ответа на гипоксию подтвердило релевантность $CoCl_2$ и производного оксихинолина в качестве индукторов гипоксии. Выявлено повышение уровня микроРНК семейства miR-374 при индукции гипоксического пути в модели трофобласта. Наблюдаемые изменения сопровождались снижением экспрессии мРНК гена *FOXM1*, которая служит мишенью для hsa-miR-374a-5p и hsa-miR-374b-5p. Данные микроРНК могут конкурировать за места связывания в мРНК *FOXM1* с hsa-miR-21-5p. Участие гена *FOXM1* в регуляции инвазивного потенциала клеток позволяет предположить роль микроРНК miR-374 и *FOXM1* в патогенезе нарушения инвазии трофобласта при формировании плаценты как предпосылки к развитию задержки роста плода и преэклампсии.

Ключевые слова: плацента, хориокарцинома, BeWo, гипоксия, кобальт, оксихинолин, *FOXM1*, микроРНК, miR-374a-5p, miR-374b-5p

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The maintenance of homeostasis during fetal development is essential for a healthy pregnancy. In pregnancy, trophoblast cells replace the endothelial lining of uterine spiral arteries, making them insensitive to systemic tone regulators and thus ensuring a constant blood flow to the placenta and the fetus. Inflammation and severe hypoxia impair trophoblast invasion [1], which is believed to be one of the major causes of preeclampsia, a severe pregnancy complication. MicroRNA (miRNA) secreted by trophoblast cells in the setting of hypoxia may be markers of impending preeclampsia and, at the same time, a pathogenic component of this condition due to their role as regulators of gene expression [2–4].

In vitro trophoblast models employ human choriocarcinoma cell lines, like BeWo b30: they are not susceptible to contact inhibition, can form confluent cell layers, have high motility [5], and therefore can be used as both villous or extravillous trophoblast models. The use of microfluidic platforms allows researchers to bring a trophoblast model closer to the actual conditions inside the organism [6, 7].

The activation of the hypoxia signaling pathway is associated with the transcription of hypoxia-inducible factor (HIF) targets. One of the classical ways to model chemical hypoxia is through exposure of the cell to cobalt (II) chloride, a compound that causes an elevation of HIF levels in the cell [8]. Derivatives of 8-oxyquinoline (OD), too, are capable of activating the hypoxia pathway by stabilizing HIF in the cell [9]. OD effects on BeWo b30 cells imitate the effects of hypoxia in the placental trophoblast [10].

The aim of this study was to analyze miRNA profiles and the expression of miRNA target genes in a model of trophoblast hypoxia.

METHODS

BeWo b30 cells were kindly provided by Prof. Dr. Christiane Albrecht (University of Bern, Switzerland) with permission from Prof. Dr. Alan Schwartz (Washington University in St. Louis, USA). The obtained cells were grown in 6-well plates, growth area 9.6 cm^2 (Corning; USA) in Gibco DMEM, high glucose (Thermo Fisher Scientific; USA) supplemented with 10% Gibco FBS One Shot (Thermo Fisher Scientific; USA), 1% Gibco MEM NEAA (100X) and 1% Gibco Pen Strep (100X). Upon reaching 80% confluence, the cells were transferred to a fresh medium that did not contain any chemical inducers of hypoxia or to a medium supplemented with 5 μM OD 4896-3212 (ChemRar High-Tech Center; Russia) or with 300 μM cobalt (II) chloride (Sigma-Aldrich; USA). After 24 h, the cells were lysed in a Qiazol Lysis Reagent (Qiagen; Germany), and RNA was extracted by means of phenol-chloroform extraction [11] using a miRNeasy Mini Kit (Qiagen; Germany). RNA concentrations were measured with a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific; USA). RNA quality control was performed in an Experion automated electrophoresis system (Bio-Rad; USA). RNA Quality Indicator (RQI) was at least 9 for all the trialed samples.

Libraries for next generation sequencing (NGS) were prepared using an NEBNext Multiplex Small RNA Library Prep Kit for Illumina (New England Biolabs; USA) and Illumina Stranded mRNA Library Prep Kit (Illumina; USA). The libraries were then sequenced in a NextSeq 500 System (Illumina; USA).

To analyze the expression of individual genes, 500 ng total RNA was reverse-transcribed using an M-MLV RT Kit (Evrogen; Russia). The obtained DNA was amplified by real-time PCR using qPCRmix-HS SYBR reagents (Evrogen; Russia). Differences in mRNA and miRNA expression between BeWo b30 cultures exposed and unexposed to hypoxia inducers were analyzed using Student's *t*-test and the Benjamini–Hochberg procedure for multiple comparisons to control the False Discovery Rate (FDR). Differential expression analysis was conducted using DESeq2 v1.28.1 [12] Differences in the expression of highly represented miRNA and mRNA were considered significant at FDR-$p < 0.05$ and log2 fold change not less than 0.4 [13].

RESULTS

The analysis of publications investigating the effects of hypoxia on the transcriptome of various cells allowed to identify a number of key genes involved in the cell response to hypoxia [14]. The expression of these genes in BeWo b30 cells exposed to OD and $CoCl_2$ was estimated from NGS data (Table 1). The expression of all genes listed in Table 1 changed significantly ($p < 0.05$), except for the genes *CDKN1A* and *ENO1* in the cells exposed to OD and the genes *SLC2A1* and *TMEM45A* in the cells exposed to $CoCl_2$. Interestingly, the expression of the *EPO* gene, which encodes erythropoietin, decreased in BeWo b30 cells exposed to cobalt, whereas under true hypoxia with low oxygen levels its expression increases [14], just like in BeWo b30 cells exposed to OD.

To verify the results of sequencing and confirm the activation of key hypoxia-responsive genes, the expression of *BNIP3*, *SLC2A3*, *PDK1* and *VEGFA* in BeWo b30 cells was evaluated by PCR. *ACTB* and *GUSB* were used as reference genes. PCR confirmed that the expression of the listed genes had been activated (Table 2).

Among highly expressed miRNAs accounting for over 95% of all BeWo b30 miRNAs, those miRNAs were identified whose level had changed significantly in the setting of chemically induced hypoxia. Those included 7 miRNAs expressed in BeWo b30 cells exposed to OD (Table 3) and 16 miRNAs expressed in BeWo b30 cells exposed to $CoCl_2$ (Table 4); only 2 miRNAs (hsa-miR-374a-5p and hsa-miR-374b-5p) occurred in both lists.

We analyzed the expression of hsa-miR-374a-5p and hsa-miR-374b-5p targets in BeWo b30 cells exposed to OD and $CoCl_2$. An earlier study reports that hsa-miR-374b-5p can regulate *FOXM1* expression in SiHa cervical cancer cells [15]. In our study, *FOXM1* expression in BeWo b30 cells decreased significantly after exposure to OD and $CoCl_2$ (1.7 and 2.6-fold, respectively). Previously, it was shown that hsa-miR-21-5p can caused a decline in *FOXM1* expression [16]; so, we analyzed the seed regions of hsa-miR-21-5p, hsa-miR-374a-5p and hsa-miR-374b-5p, to reveal that each of these 3 miRNAs has only one binding site in the 3'-untranslated FOXM1 mRNA region (see the Figure).

DISCUSSION

Cell responses to hypoxia may vary, which shows in the activation of different genes and in the varying degrees of such activation. An earlier literature analysis identified a number of key genes activated in all cells exposed to hypoxia [14]. The expression of those genes was also elevated in our BeWo b30 cells exposed to OD and $CoCl_2$, suggesting the activation of the HIF pathway. There are reports that BeWo cells exposed to $CoCl_2$ overexpress glucose transporter 1 (GLUT1) encoded by the *SLC2A1* gene [17–19]. These reports are consistent with our findings. Interestingly, the expression of the *EPO* gene, which encodes erythropoietin, declined in BeWo b30 cells exposed to $CoCl_2$, whereas true hypoxia with low oxygen levels causes an increase in *EPO* expression [14], just like in

Table 1. Expression of key genes involved in response to hypoxia in BeWo b30 cells (based on sequencing data)

Gene	log_2 fold change	
	Exposure to OD	Exposure to $CoCl_2$
DDIT4	3.5	3.6
KDM3A	1.8	3.6
BNIP3	2.3	3.3
NDRG1	3.4	3.2
SLC2A3	3.1	2.8
BHLHE40	2.8	2.6
P4HA1	2.9	2.5
PDK1	1.7	2.3
ANKRD37	3.2	2
VEGFA	1.6	1.9
ERO1A	1.7	1.9
ALDOC	4.4	1.8
CDKN1A	0.6*	1.3
STC2	1.3	1.1
ENO1	0.6*	1
SLC2A1	1.4	0.7*
TMEM45A	2	0.1*
EPO	1.4	–1.5

Note: * — designates FDR-p > 0.05; for other genes, FDR-p < 0,05.

BeWo b30 cells exposed to OD. It was shown previously that HIF can directly increase *EPO* expression in BeWo cells [20]. In another study, the level of *EPO* expression in BeWo cells was undetectable, which did not allow the researchers to assess how $CoCl_2$ and hypoxia affected its expression [21]. Cobalt is known to stimulate erythropoietin expression in the kidneys [22]. *EPO* expression is controlled by HIF-2α, and though cobalt generally induces both HIF-1α, and HIF-2α, it did not affect *EPO* expression in liver cancer cell lines Huh7 and HepG2 [23]. Perhaps, the expression of this gene may be dependent not only on the activation of the HIF signaling pathway but also on other tissue-specific factors.

Exposure of trophoblast cells to hypoxia can induce the release of certain molecules, including hypoxia-associated miRNA [24]. MiRNA released by the cell can affect the neighboring cells; shifts in miRNA expression may determine the scope of miRNA effects. However, microRNA concentrations in the producing cell have to be sufficiently for such effects to occur. Therefore, we selected 10% of miRNAs that were present in the highest concentrations in the BeWo b30 culture and then identified those whose expression had changed significantly following BeWo b30 exposure to OD or $CoCl_2$. Interestingly, of all miRNAs whose expression had significantly changed after exposure to OD or $CoCl_2$ (7 and 16 miRNAs respectively) only 2 (hsa-miR-374a-5p and hsa-miR-374b-5p) responded with overexpression to both hypoxia inducers. These 2 miRNAs are encoded in the X-chromosome in the introns of the *FTX* gene that codes for the long non-coding RNA participating in the inactivation of the X-chromosome. The miR-374 family members participate in the regulation of cell

Table 2. Expression of key genes involved in response to hypoxia in BeWo b30 cells (based on PCR data)

Gene	Linear fold change*	
	Exposure to OD	Exposure to $CoCl_2$
BNIP3	3.7	4
SLC2A3	2.2	3.6
PDK1	1.7	2.4
VEGFA	1.6	3.1

Note: * — FDR-p < 0,05.

Table 3. MiRNA with significantly changed expression after exposure to OD

miRNA	log_2 fold change	FDR-p
hsa-miR-96-5p	↓0.5	1.8×10^{-3}
hsa-miR-21-5p	↑0.4	2.7×10^{-2}
hsa-miR-429	↑0.5	4.2×10^{-4}
hsa-miR-374b-5p	↑0.5	6.6×10^{-3}
hsa-miR-374a-5p	↑0.5	1.4×10^{-3}
hsa-miR-26b-5p	↑0.6	8.0×10^{-8}
hsa-miR-181a-2-3p	↑0.7	3.8×10^{-7}

Table 4. MiRNA with significantly changed expression after exposure to $CoCl_2$

miRNA	log_2 fold change	FDR-*p*
hsa-miR-1260b	↓0.7	9.8×10^{-9}
hsa-miR-4521	↓0.7	1.6×10^{-11}
hsa-miR-148a-3p	↓0.7	8.5×10^{-8}
hsa-miR-425-5p	↓0.5	4.1×10^{-12}
hsa-miR-378i	↓0.5	6.6×10^{-10}
hsa-miR-32-5p	↓0.4	2.5×10^{-4}
hsa-miR-151a-3p	↓0.4	2.1×10^{-5}
hsa-miR-200a-3p	↑0.4	2.5×10^{-5}
hsa-miR-126-3p	↑0.4	4.0×10^{-7}
hsa-miR-484	↑0.4	9.5×10^{-8}
hsa-miR-181a-5p	↑0.5	5.2×10^{-11}
hsa-miR-25-3p	↑0.5	7.8×10^{-11}
hsa-miR-27a-3p	↑0.5	1.2×10^{-12}
hsa-miR-320a-3p	↑0.5	7.0×10^{-11}
hsa-miR-374a-5p	↑0.6	6.1×10^{-6}
hsa-miR-374b-5p	↑0.6	5.1×10^{-7}

proliferation and differentiation, growth and carcinogenesis [25]. Hsa-miR-374a-5p and hsa-miR-374b-5p have a very similar sequence; the seed-regions of these molecules are the same, suggesting that they target the same genes. It was reported that hsa-miR-374a-5p concentrations were elevated in the blood of women who delivered prematurely [26] or gave birth to babies with small gestational weight [27]; this might indicate a potential association between hypoxia and placental pathology. Under hypoxic conditions, villous trophoblast changes its metabolism from aerobic to anaerobic; this reduces oxygen consumption but increases the need for glucose. As a result, the fetus receives more oxygen but less nutrition, which might lead to intrauterine growth restriction and premature delivery [28]. A similar effect was observed in BeWo b30 cells exposed to another OD [10].

FOXM1 was identified as an hsa-miR-374a-5p and hsa-miR-374b-5p gene target; this gene encodes a transcriptional factor. The levels of its mRNA in BeWo b30 cells declined following exposure to OD and $CoCl_2$. An earlier study demonstrated that hsa-miR-21-5p was capable of reducing *FOXM1* expression

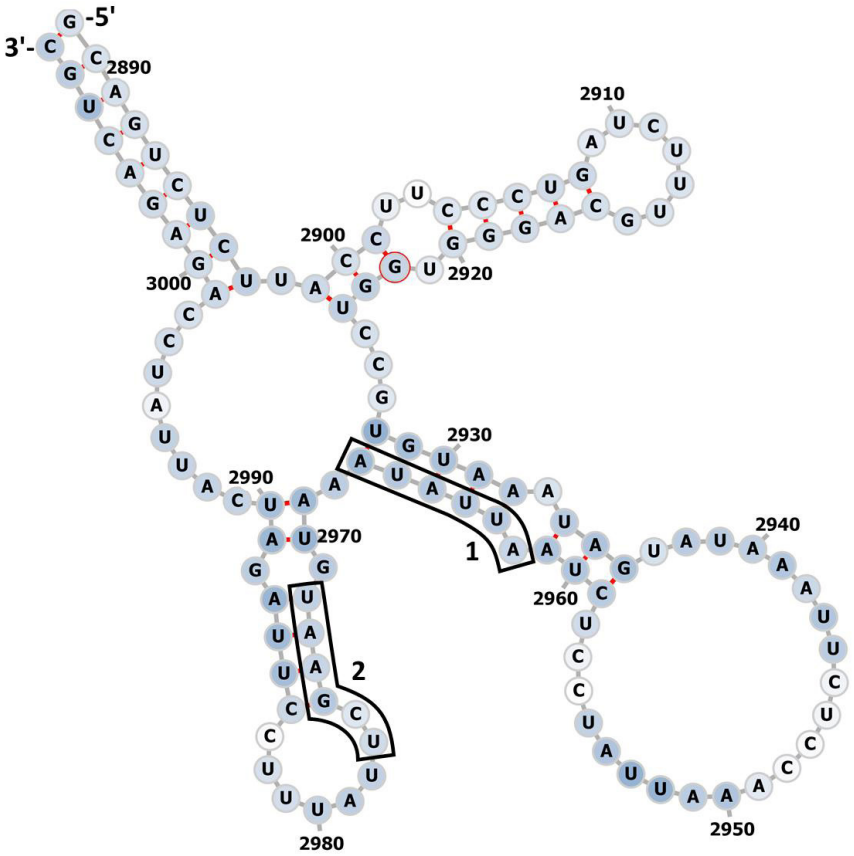

Fig. Positions of microRNA binding sites on a fragment of a 3'-untranslated region of *FOXM1* mRNA (transcription variant 2). Nucleotide numbers are represented by 4-digit numbers. 1 — binding sites for hsa-miR-374a-5p and hsa-miR-374b-5p; 2 — a binding site for hsa-miR-21-5p

and proliferation of HTR8/SVneo choriocarcinoma cells; in the setting of preeclampsia, hsa-miR-21-5p levels were increased and the expression of *FOXM1* was decreased in the placenta [16]. These reports are in good agreement with our findings. At the same time, a significant elevation of hsa-miR-21-5p concentrations in BeWo b30 cells was observed only after exposure to OD, as opposed to $CoCl_2$, suggesting the involvement of hsa-miR-374a-5p and hsa-miR-374b-5p in the regulation of *FOXM1* expression in our trophoblast model. According to the spatial principle, the proximity of binding sites implies competition between hsa-miR-21-5p and hsa-miR-374a-5p/hsa-miR-374b-5p, because in order for the complex of argonaute proteins with these miRNAs to interact with *FOXM1* mRNA, a significant steric strain is needed in the target mRNA sequence (see the Figure).

Impaired trophoblast invasion of the uterine wall and spiral arteries in the setting of preeclampsia might be explained by the reduced expression of *FOXM1* governed by hsa-miR-374b-5p, as was previously demonstrated for SiHa cervical cancer cells [15]. At < 3% oxygen level simulating physiological hypoxia, *FOXM1* expression in JEG-3 choriocarcinoma cells was initially high. But as the level of oxygen was falling, so was the expression of the gene. *FOXM1* knockdown suppressed JEG-3 cell migration, and the culture medium in which the cells had been grown inhibited angiogenesis in the culture of endothelial cells (HUVEC) [29].

CONCLUSIONS

Our study demonstrates that exposure of BeWo b30 cells to oxyquinoline derivatives and cobalt (II) chloride may be used as a trophoblast hypoxia model. This was confirmed by the activation of key hypoxia-responsive genes. At the same time, the response of BeWo b30 cells to hypoxia manifesting in the changed miRNA expression varied significantly depending on the compound used to induce hypoxia. Both cobalt and the oxyquinoline derivative caused an increase in the expression of miR-374, suggesting its participation in response to hypoxia. The reduced expression of *FOXM1*, the gene target for the miR-374 family, suggests the role of miR-374 and *FOXM1* in the pathogenesis of impaired trophoblast invasion during placental development as a prerequisite for intrauterine growth restriction and preeclampsia.

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CHANGES IN GUT MICROBIOTA COMPOSITION AND THEIR ASSOCIATIONS WITH CORTISOL, MELATONIN AND INTERLEUKIN 6 IN PATIENTS WITH CHRONIC INSOMNIA

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The relationship between the gut microbiota and chronic insomnia remains understudied. The aim of this paper was to investigate changes in the taxonomic composition of the gut microbiota and their associations with the levels of cortisol, melatonin and IL6 in patients with chronic insomnia. Our comparative prospective cross-sectional study enrolled 55 patients with chronic insomnia, who formed the main group (female patients: 58.2%, male patients: 41.8%; mean age 31.6 ± 7.4 years), and 50 healthy volunteers, who comprised the control group (females: 68.0%, males: 32.0%; mean age 33.2 ± 6.6 years). The taxonomic composition of the gut microbiota was profiled using 16S rRNA gene sequencing. Plasma cortisol and IL 6 and urine melatonin were measured by means of ELISA. Sleep quality was evaluated using the Pittsburgh Sleep Quality Index (PSQI). In patients with chronic insomnia, the abundance of Faecalibacterium ($p = 0.048$), Prevotella 9 ($p < 0.001$) and Lachnospira ($p = 0.036$) was lower, whereas the abundance of Blautia ($p = 0.012$) and Eubacteriumhallii ($p = 0.003$) was higher than in healthy volunteers. Significant correlations were established between the levels of IL6 and the abundance of Faecalibacterium ($r = –0.44$; $p = 0.001$) and Blautia ($r = 0.42$; $p < 0.001$), as well as between cortisol concentrations and the abundance of Lachnospira ($r = –0.41$; $p = 0.048$). The abundance of Faecalibacterium and Blautiac was correlated with higher PSQI ($r = –0.47$, $p = 0.001$; $r = 0.45$, $p < 0.001$, respectively). Our study contributed to the pool of data about changes in the gut microbiota and their associations with some endocrine and inflammation markers in patients with chronic insomnia. These data can be exploited to propose new strategies for the diagnosis and personalized treatment of insomnia aimed at normalizing the patient's gut microbiota.

Keywords: insomnia, gut microbiota, gut-brain axis, cortisol, melatonin, IL6

Author contribution: Masyutina AA, Fatovenko YuV collected, analyzed and interpreted the obtained data; Gumenyuk LN proposed the concept and design for the study; Sorokina LE, Bayramova SS, Alekseenko AA performed statistical analysis; Shavrov YuV, Romanova AA, Seydametova DI wrote the manuscript.

Compliance with ethical standards: the study was approved by the Ethics Committee of Georgievsky Medical Academy (Protocol № 10 dated November 16, 2020) and complied with the Declaration of Helsinki. Voluntary informed consent was obtained from all study participants.

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ИЗМЕНЕНИЯ МИКРОБИОТЫ КИШЕЧНИКА И ИХ СВЯЗЬ С КОРТИЗОЛОМ, МЕЛАТОНИНОМ И ИНТЕРЛЕЙКИНОМ-6 У ЛИЦ С ХРОНИЧЕСКОЙ ИНСОМНИЕЙ

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На сегодняшний день актуальной остается проблема взаимосвязи микробиоты кишечника и хронической инсомнии. Целью исследования было изучить изменения таксономического состава микробиоты кишечника и характер их взаимосвязи с кортизолом, мелатонином и IL6 у лиц с хронической инсомнией. В одномоментном сравнительном проспективном исследовании приняли участие 55 лиц с хронической инсомнией (основная группа: женщины — 58,2%, мужчины — 41,8%; средний возраст — 31,6 ± 7,4 лет) и 50 лиц без инсомнии (контрольная группа: женщины — 68,0%, мужчины — 32,0%; средний возраст — 33,2 ± 6,6 лет). Оценивали таксономический состав микробиоты кишечника методом секвенирования гена 16S rRNA. Определяли уровни кортизола и IL6 в плазме крови, мелатонина в моче с помощью иммуноферментного анализа. Для оценки качества сна использовали опросник PSQI. У лиц с хронической инсомнией обнаружены статистически значимое снижение численности *Faecalibacterium* ($p = 0{,}048$), Prevotella 9 ($p < 0{,}001$) и *Lachnospira* ($p = 0{,}036$) и повышение численности *Blautia* ($p = 0{,}012$) и *Eubacteriumhallii* ($p = 0{,}003$). Установлены статистически значимые корреляции значений IL6 с уровнем бактерий *Faecalibacterium* ($r = –0{,}44$; $p = 0{,}001$) и *Blautia* ($r = 0{,}42$; $p < 0{,}001$), концентрации кортизола и уровня бактерий *Lachnospira* ($r = –0{,}41$; $p = 0{,}048$). Выявлена сопряженность уровня бактерий *Faecalibacterium*, *Blautiac* с более высокими баллами по опроснику PSQI ($r = –0{,}47$, $p = 0{,}001$; $r = 0{,}45$, $p < 0{,}001$ соответственно). Таким образом, получены дополнительные данные об особенностях изменений микробиоты кишечника и их связи с некоторыми гормональными и воспалительными биомаркерами при хронической инсомнии, позволяющие применять новые терапевтические стратегии в персонифицированном лечении и диагностике инсомнии, направленные на нормализацию кишечной микробиоты.

Ключевые слова: инсомния, микробиота кишечника, ось «микробиота–кишечник–мозг», кортизол, мелатонин, IL6

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Insomnia is common in the general population and has multiple medical and social consequences [1, 2]. Today, insomnia is considered to be a psychobiological disorder associated with psychological, neuroendocrine, neuroimmune, electrophysiological, neurophysiological, structural, and functional changes in the body [3]. There is extensive discussion in the literature about the role of some biomarkers like cortisol [4], melatonin [5] and IL6 [6] in insomnia.

In recent years, there has been increasing evidence of the close interplay between the gut microbiota and the central nervous system; it occurs through the microbiota-gut-brain axis and has the central role in neuroimmunoendocrine interactions [7, 8]. A number of experimental and clinical studies have demonstrated a relationship between the gut microbiota and sleep. For example, an interference with the normal sleep pattern of laboratory mice caused changes to the composition and diversity of their gut microbiota [9]. Similar changes were observed in the gut microbiota of shift workers [10]. Another study reports that therapy for intestinal dysbiosis increases sleep duration and improves sleep efficiency [11–12]. However, many aspects of the relationship between the gut microbiota and inflammation/endocrine markers in the setting of chronic insomnia remain understudied. There are no published data on the association between the gut microbiota and cortisol levels in patients with chronic insomnia.

According to some publications, insomnia induces a systemic hypoergic inflammatory response characterized by elevated proinflammatory cytokines. One of such cytokines is IL6; it is recognized by some authors as an important somnogenic factor [13]. Elevated serum IL6 has been negatively correlated with the self-reported quality of sleep in insomniacs [6]. A few studies point to an association between gut dysbiosis and the levels of IL6 [13, 14]. However, the impact of microbiota composition on IL6 concentrations is not transparent due to conflicting data. For example, the abundance of *Proteobacteria* has been positively correlated with IL6 levels in insomniac men [13]. In another study, an association has been established between plasma IL6 concentrations and the abundance of *Faecalibacterium* and *Blautia* in the gut microbiota of patients with chronic insomnia [14]. So, the relationship between the gut microbiota and chronic insomnia requires further elucidation.

The aim of this study was to investigate the taxonomic composition of the gut microbiota and their associations with the levels of cortisol, melatonin and IL6 in patients with chronic insomnia.

METHODS

This study was a comparative prospective cross-sectional study. Using the continuous sampling method, we selected 55 patients of the Somnology Center, Simferopol, with previously diagnosed chronic insomnia (the main group) and 50 healthy volunteers without insomnia (the control group) who presented for an annual medical checkup at the Hemocode medical center, Simferopol, and were eligible for the study. The main group consisted of 32 female (58.2%) and 23 male (41.8%) patients; the mean age in this group was 31.6 ± 7.4 years. The control group (CG) included 34 healthy females (68.0%) and 16 males (32.0%); their mean age was 33.2 ± 6.6 years. The groups were comparable in terms of sex ($p = 0.95$; χ^2), age ($p = 0.91$; χ^2) and body mass index ($p = 0.055$; χ^2).

Inclusion criteria for patients: chronic insomnia lasting over 3 months, age of 18–45 years.

Exclusion criteria for patients: body temperature above 36.9 °C; types 1 or 2 diabetes mellitus; obesity; myocardial infarction; severe arrythmias; congestive heart failure; hypertensive heart disease; a past history of stroke or TIA; stroke within 6 months preceding the study; severe or decompensated comorbidities that could interfere with the patient's participation in the study or affect the outcome of the study; irritable bowel syndrome; chronic gastrointestinal or hepatic disorders; blood disorders; cancer; bacterial or viral infection; mycosis; constipation or diarrhea in the month preceding the study; psychiatric disorders; a past history of alcoholism or substance abuse; therapy with antibiotics, prebiotics, probiotics, symbiotics, antisecretory drugs, psychotropic drugs or any other medications capable of affecting sleep within 3 months preceding the study; intake of drugs that affect colonic function in the month preceding the study; psychotherapy for insomnia within 3 months preceding the study; shift work or working across time zones in the month preceding the study.

Inclusion criteria for healthy volunteers: age of 18–45 years; no depression according to the Patient Health Questionnaire-9 (PHQ-9 score < 5 points); no anxiety according to PHQ and Generalized Anxiety Disorder-7 scale (< 5 points); no somatoform disorders (PHQ-15 < 5 points); no chronic conditions or allergies; no infections or acute conditions within 2 months preceding the study; no constipation or diarrhea in the month preceding the study; no shift work or jobs involving travelling across time zones in the month preceding the study; no therapy with prebiotics, probiotics or symbiotics within 3 months preceding the study; no therapy affecting colonic function in the month before the study; no past history of psychiatric disorders, alcoholism or drug addiction; voluntary informed consent to participate.

Exclusion criteria for healthy volunteers: body temperature above 36.9 °C; shift work or jobs involving travelling across time zones in the month preceding the study.

Insomnia was diagnosed according to the criteria of the International Classification of Sleep disorders, ver. 3 (2014) [15]. The quality of sleep was assessed using the Pittsburgh Sleep Quality Index (PSQI) [16]. The severity of insomnia was assessed using the Insomnia Severity Index (ISI) [16].

To analyze the taxonomic composition of the gut microbiota, stool samples were collected in the morning (from 8.00 to 11.00 am), then frozen and stored in disposable plastic containers at –80 °C until further use for the metagenomic analysis. Total DNA extraction was performed by means of phenol extraction. The nucleotide sequence of the extracted DNA was determined by shotgun sequencing; the procedure was carried out using a high-throughput SOLiD5500 Wildfire instrument (Applied Biosystems; USA) [17].

The obtained reads were filtered and taxonomically classified in QIIME ver. 1.9.1 (Caporaso labs; USA) [18]. The approach employed for taxonomic profiling involved the use of 2 taxonomic databases. First, a reference dataset of operational taxonomic units (OUTs) was selected by comparing the yielded 16S rRNA reads with GreenGenes data, ver. 13.5 [19]. Then, the RDP algorithm was applied to assign an appropriate taxonomic group to the OUT data using the human intestinal microbiota 16S rRNA database (HITdb) as a reference [20].

The abundance and diversity of the patients' gut microbiota was studied through the identification of microbial species, genera and phyla. The α-diversity of the microbial community was assessed based on the values of Chao1, Sobs (the number of species observed in the studied sample) and ACE (Abundance-based Coverage Estimator); all calculations were performed in Mothur v.1.22.0 (http:// www.mothur.org).

Serum levels of cortisol and IL6 were measured using ELISA assays (Vector-Best; Novosibirsk; Russia). Fasting peripheral

blood was collected in the morning (7:00–9:00) after at least 15 minutes of rest.

Melatonin production was measured from the excretion of its major metabolite, 6-sulfatoxymelatonin (aMT6s) in night (6:00) and day (20:00) urine using ELISA assays (Buhlmann; Switzerland). The samples were processed in a semi-automated StatFax 2100 analyzer (Awareness Technology; USA). Test tubes containing the collected blood and urine samples were stored at –20 °C.

Statistical analysis was carried out in STATISTICA 8.0 (StatSoft. Inc.; USA). For normally distributed variables, means and standard deviations were calculated; for non-normally distributed variables, medians, Q1 and Q3 values were determined. The normality of data distribution was tested using a Gaussian distribution test. For categorical variables, percentages and absolute quantities were calculated. Normally distributed quantitative variables were compared using the parametric Student's t-test. For non-normally distributed variables, the Mann–Whitney U-test was applied. Categorical variables were compared using the chi-square test. To assess the correlation between the studied variables, Spearman's correlation coefficient was applied. Differences were considered significant at $p < 0.05$. In addition, correlation analysis and multiple rank correlation analysis were carried out; the validity of correlations was tested using tables of critical values.

RESULTS

Participant characteristics are provided in Table 1. In the main group, the duration of insomnia varied from 5 months to 3.5 years and was 1.7 [1.1; 2.4] years on average. Twenty-one patients (38.6%) from the main group had a past history of therapy for insomnia. Of them, plant-derived sedatives had been tried by 10 patients (47.6%); 8 (38.1%) patients had tried melatonin-based medications and 3 patients (14.3%) had undergone psychotherapy.

While studying the taxonomic composition of the gut microbiota, we found the α-diversity of the microbial community was much poorer in patients with chronic insomnia than in healthy volunteers (based on Chao1; $p = 0.016$). ACE and Sobs values were slightly lower in the main group but these differences were insignificant ($p = 0.054$ and $p = 0.052$, respectively; Fig. 1). However, the groups differed significantly in terms of Actinobacteria abundance: these microorganisms were more abundant in patients with chronic insomnia than in healthy volunteers ($p = 0.0003$).

At the genus level, the gut microbiota of patients with insomnia was characterized by a significant reduction in the abundance of *Faecalibacterium* ($p = 0.048$), *Prevotella 9* ($p = 0.0002$) and *Lachnospira* ($p = 0.036$), as compared to the control group, and a more abundant population of *Blautia* ($p = 0.012$) and *Eubacterium hallii* ($p = 0.003$) (Fig. 2).

The serum levels of IL6 and cortisol were significantly higher in patients with insomnia than in the control group. Melatonin levels were lower in the control group, but the differences between the groups were insignificant (Table 2).

The analysis of the relationship between the composition of the gut microbiota and inflammation/endocrine markers revealed significant correlations between IL6 levels and the abundance of *Faecalibacterium* ($r = –0.44$; $p = 0.001$) and *Blautia* ($r = 0.42$; $p < 0.001$), as well as between cortisol levels and the abundance of *Lachnospira* ($r = –0.41$; $p = 0.048$).

The correlation analysis revealed a correlation between the abundance of *Faecalibacterium* and *Blautia* and higher PSQI ($r = –0.47$, $p = 0.001$; $r = 0.45$, $p < 0.001$, respectively) and higher ISI scores ($r = –0.51$, $p = 0.002$; $r = 0.48$, $p < 0.001$, respectively). The abundance of *Faecalibacterium* was negatively correlated with higher depression scores ($r = –0.44$; $p < 0.001$), whereas the abundance of *Lachnospira* was negatively correlated with higher anxiety scores ($r = –0.51$; $p < 0.001$) and higher ISI ($r = –0.52$; $p < 0.001$).

Significant correlations were established between the levels of IL6 and the abundance of *Faecalibacterium* ($r = –0.44$; $p = 0.001$) and *Blautia* ($r = 0.42$; $p < 0.001$). In addition, we were able to establish a correlation between the abundance of some bacteria (*Faecalibacterium*, *Blautia*) and the quality of sleep on the PSQI scale ($r = 0.37$; $p = 0.001$; $r = 0.54$; $p = 0.011$, respectively). The severity of insomnia (the total ISI score) was directly correlated with the abundance of these bacteria ($r = 0.67$; $p = 0.005$; $r = 0.29$; $p = 0.0001$, respectively). The severity of depression on the PHQ-9 scale was correlated with the abundance of *Faecalibacterium* and *Blautia* ($r = 0.19$; $p = 0.005$; $r = 0.32$; $p = 0.003$, respectively). Cortisol levels were correlated with the abundance of *Lachnospira* and the severity of insomnia on the ISI scale ($r = 0.37$; $p = 0.002$).

Besides, a correlation was discovered between the abundance of Lachnospira and higher ISI ($r = –0.38$; $p < 0.001$) and GAD-7 scores ($r = –0.47$; $p < 0.0001$).

DISCUSSION

A promising area of research, the metabolic integration between the gut microbiota and the brain implemented via the gut-brain axis is the central element of neuroimmunoendocrine interactions [21]. Today, many researchers hold the opinion that the gut microbiota plays a significant role in psychiatric disorders and homeostatic imbalance [22, 23]. Some studies have demonstrated an association between the gut microbiota and sleep. For instance, changes to a sleeping schedule were reported to induce changes in the composition of the gut

Table 1. Characteristics of the participants with and without insomnia

Parameter	Man group ($n = 55$)	Control group ($n = 50$)
Women / men (*n*, %)	32 (58.2) / 23 (41.8)	34 (68.0) / 16 (32.0%)
Mean age, years (M ± CD)	31.6±7.4	33.2 ± 6.6
Body mass index, kg/m^2	25.2 ± 4.4	25.6 ± 3.9
PSQI	15.4 ± 3.7*	3.3 ± 1.4
ISI, points	5.3 [3.6;6.4]	13.2 [10.4; 16.7]
PHQ-9	9.1 ± 4.2*	3.4 ± 1.2
GAD-7	7.9 ± 4.5*	2.9 ± 1.3
PHQ-15	9.9 ± 3.2*	3.3 ± 1.5

Note: * — $p < 0.001$ relative to CG, ISI — Insomnia Severity Index.

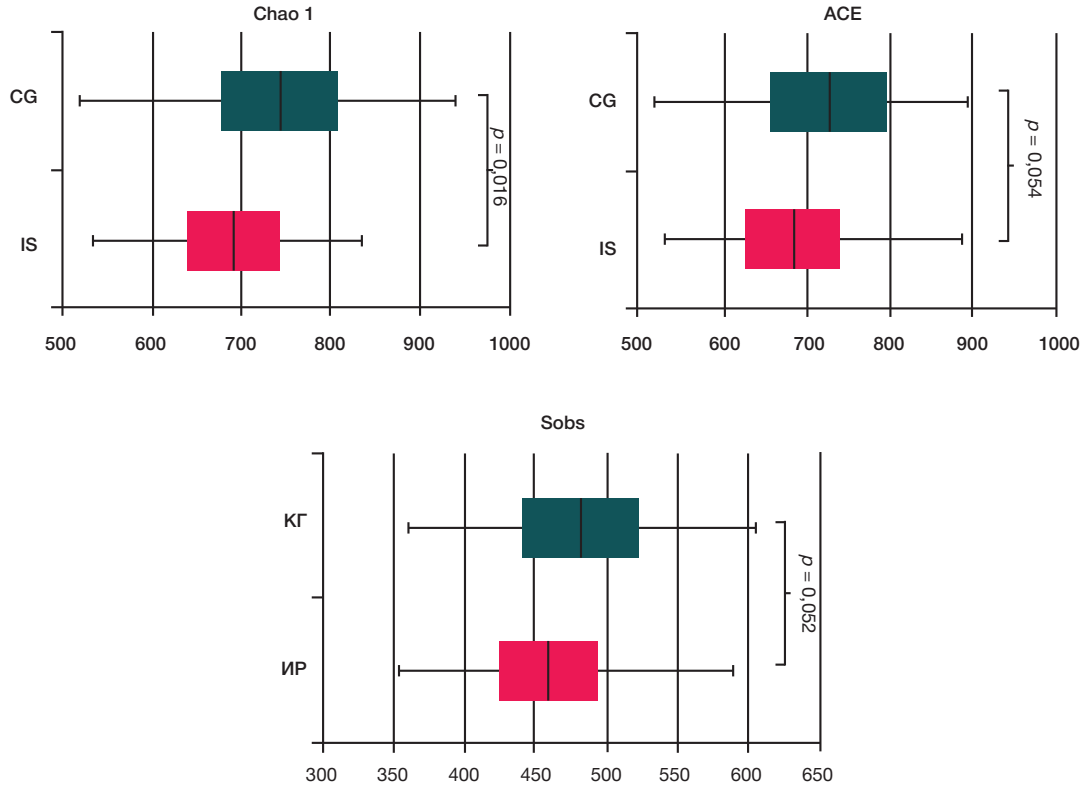

Fig. 1. Comparison of the gut microbiota composition at the phylum level between patients with insomnia and healthy volunteers. IS — insomnia, CG — control group

microbiota [9]. In another study, therapy for intestinal dysbiosis increased sleep duration and sleep efficiency [11]. At the same time, many aspects of the relationship between the gut microbiota and endocrine/inflammation markers in patients with chronic insomnia remain understudied.

Our study demonstrates that the composition of the gut microbiota differs significantly between patients suffering from chronic insomnia and healthy individuals. Specifically, the bacterial α-diversity was poorer in patients with chronic insomnia. This finding was confirmed by lower Chao1 index in such patients and is consistent with the results of an earlier study [24]. In patients with chronic insomnia, the population of anaerobic *Faecalibacterium*, *Prevotella 9* and *Lachnospira* that produce short-chain fatty acids and are known for their anti-inflammatory properties was smaller [25, 26]. At the same time, the abundance of *Blautia* and *Eubacterium hallii* was greater. These are potential pathobionts that can cause dysregulation of immune response involving the regulatory T cells (Tregs) of the intestine, anti-inflammatory IL10 and the regenerating islet-derived protein 3γ (REGIIIγ, REG3G). Our findings partially agree with the results of a study [14] in which a reduction in the relative abundance of *Lachnospira* was characteristic of patients with acute insomnia [14]. The discrepancy between the results of our study and the cited publication can be explained by differences in the applied inclusion criteria. The groups were significantly different in terms of age: 31.6 ± 7.4 years in our study vs.

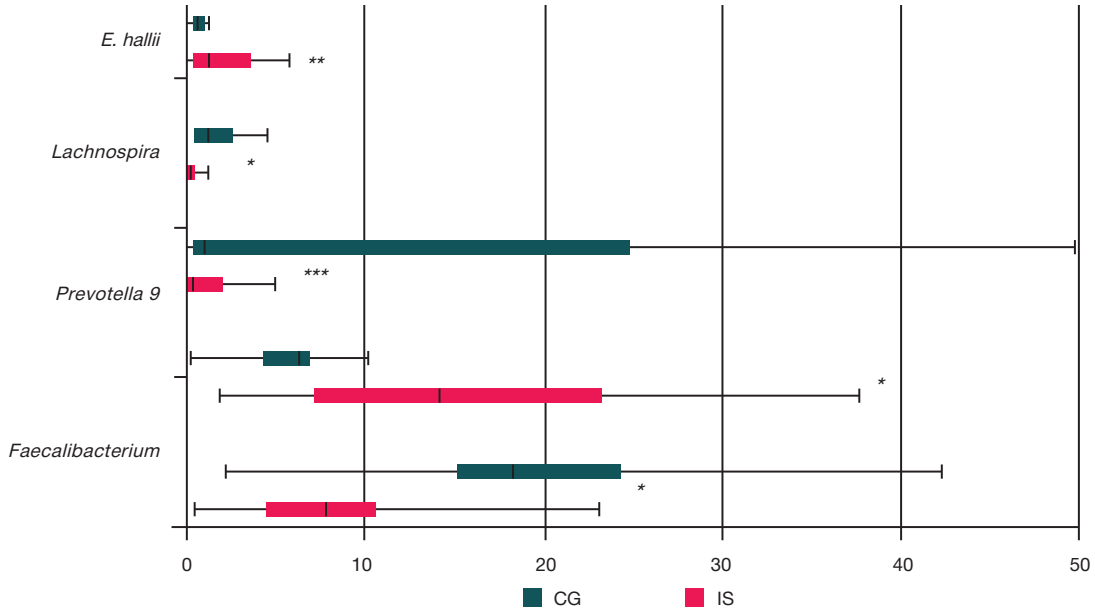

Fig. 2. Comparison of the gut microbiota composition at the genus level between patients with insomnia and healthy volunteers. IS — insomnia CG — control group

Table 2. Inflammation and endocrine markers in patients with insomnia and healthy volunteers (mean ± SD)

Parameter	Main group *n* = 55	Control group *n* = 50	*p*
Cortisol, nmol/L	581.5 ± 110.6	323.5 ± 108.1	*p* = 0.036
Melatonin (8:00), pg/ml	12.2 ± 2.4	13.8 ± 2.6	*p* = 0.652
Melatonin (20:00), pg/ml	3.5 ± 1.2	4.4 ± 1.1	*p* = 0.581
IL6, pg/ml	5.6 ± 0.9	2.8 ± 0.8	*p* = 0.014

43.5 ± 6.9 years in the study of our colleagues. Besides, we did not include patients with psychiatric disorders in order to negate their effect on the results of the study, whereas our colleagues did not specify psychiatric disorders as an exclusion criterion.

The established correlation between the reduced abundance of *Faecalibacterium*, the increased abundance of *Blautia* and higher PSQI and ISI scores leads us to hypothesize that changes in the abundance of these bacteria are typical for patients with chronic insomnia. It is also possible that an expansion of the Faecalibacterium population and a shrinkage of the *Blautia* population induced by therapy mitigates insomnia. Further research is necessary to test this hypothesis.

The analysis of endocrine and inflammation markers revealed that serum cortisol and IL6 levels differed significantly between patients with chronic insomnia and healthy volunteers. These two biomarkers were significantly correlated with the abundance of some bacteria inhabiting the intestine, suggesting an association between sleep disorders and the composition of gut microbiota. For example, negative correlations were established between cortisol levels and the abundance of *Lachnospira.* These findings contradict the results of another study which reported the lack of significant correlations between cortisol levels and gut microbiota diversity in patients with insomnia [13]. The lack of consistency between these two sets of data might be explained by the fact that our sample included individuals of both sexes, whereas in the study of our colleagues [13] the sample was all-male. This suggests the existence of sex-related differences in the association between cortisol levels and the composition of gut microbiota. Further research is needed to elucidate this problem.

Our study did not confirm the correlation between aMT6s excretion and changes in the microbiota composition in patients with chronic insomnia. While analyzing the literature, we did not find any publications that explored this problem in a sample of patients with chronic insomnia. However, there is evidence about the role of melatonin in regulating the composition of the gut microbiota [27]. In the cited study, melatonin concentrations were measured from urine aMT6s, which, in turn, characterizes secretion of melatonin by the pineal gland [28], whereas in an earlier study [29] the authors focused on enteral melatonin in the gastrointestinal tract produced by enterochromaffin intestinal cells.

Chronic inflammation might be an important pathogenic link between the gut microbiota and sleep. For example, it has been demonstrated that chronic sleep deprivation is associated with elevated plasma IL6 [30]. In another study, sleep duration was directly correlated with plasma IL6 [31]. Intestinal dysbiosis may trigger inflammatory immune response accompanied by elevated systemic proinflammatory cytokines. Established in [14], the correlation between the levels of IL6 and the abundance of *Faecalibacterium* and *Blautiay* in patients with chronic insomnia was confirmed by our study, suggesting a significant role of these bacteria in maintaining inflammation and aggravating insomnia and its consequence. Despite the fact that the role of *Blautia* in the development of inflammation has been proved, data on their association are contradictory. Some authors report a direct correlation between the abundance of *Blautia* and inflammatory dysregulation [32], others state that the correlation is negative [33]. According to our study, an expansion in the *Blautia* population promotes increased plasma IL6. Importantly, we analyzed the level of one proinflammatory cytokine only and so cannot provide the characterization of the overall inflammatory status in patients with chronic insomnia.

CONCLUSIONS

The gut microbiota of patients with chronic insomnia is characterized by pronounced changes in its taxonomic composition and microbial abundance. Significant correlations observed between some representatives of the gut microbiota and PSQI, endocrine and inflammation markers support the concept of the association between the composition of the gut microbiota, the abundance of its members and chronic insomnia. Further research is needed to confirm the role of the gut microbiota in the pathogenesis of chronic insomnia. The association between the composition of the gut microbiota and endocrine markers remains understudied. Therapy aimed at balancing the composition of the gut microbiota might improve the efficacy of therapy for chronic insomnia.

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THE IMPACT OF THE NOVEL CORONAVIRUS INFECTION COVID-19 ON THE MOTHER-PLACENTA-FETUS SYSTEM

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Impaired placental development during early pregnancy associated with systemic damage to the vascular endothelium in patients with COVID-19 may result in a number of complications. The study was aimed to reveal histological and immunohistochemical (IHC) features of placental tissue in pregnant women with COVID-19 at different stages of gestation, and to examine the contribution of those to pathogenesis of the disease involving mother-placenta-fetus system. The following two groups of pregnant women were studied: index group of 66 patients with COVID-19, and comparison group of 40 women with no symptoms of viral infection. Macroscopic and microscopic examination, and the IHC analysis of placental samples were carried out. Clinical and anamnestic characteristics of patients with COVID-19 were analyzed taking into account disease severity, delivery route and perinatal outcome. ICH staining using primary antibody revealed elevated expression of proinflammatory factors (TNFα, IL8) and reduced level of anti-inflammatory factors (IL4) in placental structures of patients with moderate and severe COVID-19 ($p < 0.05$). The villous tree rearrangement and the development of subclinical placental insufficiency, which could in some cases be decompensated during labor, resulting in clinical manifestations of acute fetal hypoxia were detected in the placental samples obtained from the index group patients. The obstetrical tactics for mothers with COVID-19 should be decided individually based on the risk factors; continuous cardiotocography should be used during labor. It may be appropriate to conduct IHC analysis of placenta in puerperant women with COVID-19 in order to fine-tune the tactics of neonatal management and to predict possible neonatal complications.

Keywords: pregnancy, placenta, COVID-19, fetus, chronic hypoxia, placental insufficiency, proinflammatory response

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Author contribution: Nizyaeva NV — pathomorphological study and IHC analysis, systematic analysis, manuscript writing; Lomova NA — analysis of clinical data, systematic analysis, manuscript writing; Dolgopolova EL — collection and preparation of biological matrix samples in the red zone, statistical analysis of the results; Petrova UL — collection and preparation of biological matrix samples in the red zone; Karapetyan TE — analysis of clinical data; Shmakov RG — analysis of clinical data in the red zone, systematic analysis, manuscript editing; Frankevich VE — preparation of the study, systematic analysis.

Compliance with ethical standards: all patients submitted the informed consent to participate in the study; the study met the requirements of the Declaration of Helsinki, International Conference on Harmonization (ICF), Good Clinical Practice (GCP), and Federal Law No. 323-FZ "On the Basics of Protecting Citizens' Health in the Russian Federation" of November 21, 2011.

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ВЛИЯНИЕ НОВОЙ КОРОНАВИРУСНОЙ ИНФЕКЦИИ COVID-19 НА СИСТЕМУ «МАТЬ–ПЛАЦЕНТА–ПЛОД»

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Нарушение развития плаценты на ранних сроках беременности, ассоциированное с системным поражением эндотелия сосудов при COVID-19, может привести к ряду осложнений. Целью исследования было выявить гистологические и иммуногистохимические (ИГХ) особенности ткани плаценты у беременных женщин с COVID-19 на разных сроках гестации и изучить их роль в патогенетических механизмах болезни в системе «мать–плацента–плод». Исследовали две группы беременных: основную из 66 пациенток с COVID-19 и группу сравнения из 40 женщин без признаков вирусной инфекции. Выполняли макроскопическое, микроскопическое и ИГХ-исследования образцов плацент. Проведен анализ клинико-анамнестических характеристик пациенток с COVID-19 с учетом тяжести течения заболевания, родоразрешения и перинатальных исходов. При ИГХ-окрашивании первичными антителами у пациенток с тяжелым и среднетяжелым течением COVID-19 повышена экспрессия в структурах плаценты провоспалительных факторов (TNFα, IL8) и снижен уровень противовоспалительных (IL4) ($p < 0,05$). В образцах плацент женщин основной группы детектирована перестройка ворсинчатого дерева с развитием субклинической плацентарной недостаточности, в ряде случаев декомпенсируемой в родах, с развитием клинической картины острой гипоксии плода. У матерей с COVID-19 следует выбирать акушерскую тактику индивидуально с учетом факторов риска и проведением в родах непрерывной кардиотокографии. Целесообразно проводить ИГХ-исследование плаценты родильниц с COVID-19 для уточнения тактики ведения новорожденного и прогноза возможных неонатальных осложнений.

Ключевые слова: беременность, плацента, COVID-19, плод, хроническая гипоксия, плацентарная недостаточность, провоспалительный ответ

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Researchers from all over the world are actively exploring SARS-CoV-2 virus and related COVID-19 disease. These studies are of particular importance for the cohort of pregnant women because of the urgency of the challenge; new information about COVID-19 may contribute to the future health of the next generation. To date, there are no reliable reports of increased obstetric disorder prevalence, as well as of possible perinatal SARS-CoV-2 transmission. Possible correlation between COVID-19 in pregnant/puerperant women and the development of fetal distress syndrome, as well as with thrombocytopenia and liver dysfunction in newborns has been shown [1, 2]. According to researchers from Zurich, COVID-19 causes systemic inflammation of lung vasculature (vasculitis) affecting multiple organs and systems; this has no apparent connection with pneumonia. Inflammation involves endothelium, the internal lining of various organs' blood vessels [3]. Impaired placenta development during early pregnancy associated with systemic endothelial damage in patients with COVID-19 may result in oxidative stress in the placenta, and in a number of complications [4, 5]. Literature analysis shows that systemic inflammatory response syndrome (SIRS) in pregnant women with COVID-19 involves multiple pathogenetic mechanisms resulting in inflammation activation and immune response alteration. Understanding of SIRS pathophysiologal mechanisms involved in mother-placenta-fetus system in patients with COVID-19 promotes improvement of diagnostic methods and deciding pathogenetically based treatment for a number of obstetric complications [5, 6].

The study was aimed to reveal histological and immunohistochemical (IHC) features of placental tissue in pregnant women with COVID-19 at different stages of gestation, and to examine the contribution of these abnormalities to pathogenesis of the disease involving mother-placenta-fetus system.

METHODS

From March to May 2020 in the 1st Infectious Diseases Department of the National Medical Research Center for Obstetrics, Gynecology and Perinatology named after Academician V. I. Kulakov the "red zone" was established for examination and treatment of patients with COVID-19, among them of pregnant women. A total of 190 beds for patients with COVID-19 and 60 beds for obstetric patients were prepared. Biological material samples (maternal venous blood, cord blood, amniotic fluid, placental tissue) were deposited in the certified biobank of the Center. The index group included pregnant women with verified COVID-19 diagnosis. The average age of the patients was 30.3 ± 6.25 years. The patients were enrolled once they were admitted. In order to attain the envisaged goals, clinical and laboratory investigation was performed, and pregnancy management was provided in 66 women with COVID-19 (placental tissue was assessed in 42 cases that ended in delivery, and in one fatal case), and 40 pregnant women of comparison group with no clinical manifestations and laboratory signs of viral infection.

Inclusion criteria: group I — COVID-19 confirmed by PCR test; group II — no COVID-19 based on physical examination data and PCR test. Exclusion criteria: multiple pregnancy.

Macroscopic and microscopic examination was carried out of 42 placental tissue samples obtained from puerperant women with COVID-19, and one dead patient, as well as of 40 placental tissue samples obtained from healthy puerperant women of the comparison group. Paraffin-embedded placental tissue blocks taken from women with uncomplicated pregnancy who delivered in 2017–2018 were used as additional comparison group and to exclude the suppressed disease. Histological assessment (hematoxylin and eosin staining) and IHC analysis of serial sections were performed. ICH analysis of 10% formaline (pH 7.4) fixed 4-μm paraffin sections was carried out using Ventana immunostainer (Roche; UK) with closed detection kit. The automated staining protocol included all stages of standard IHC staining. Ultra View Universal DAB Detection Kit (Ventana Medical Systems, Inc.; USA) was used for visualization. Primary monoclonal SARS-CoV-2 nucleoprotein antibody (N-protein (NP), clon 1518 (1:1000); Bialexa, Russia) was used for detection of viral particles. The control of NP antibody to SARS-CoV-2 expression assessment was the most important issue. Antibodies to SARS-CoV-2 were previously checked by immunoblotting techniques with the use of recombinant protein. Morphometry was used to evaluate the degree of the villous tree syncytiotrophoblast membrane damage. Assessment was carried out with NIS-Element AR3 image analysis system (Nikon; Czech Republic) for Nikon ECLIPSE 80i microscope. Statistical data processing was performed using the SPSS Statistics ver. 21 software package for Windows.

RESULTS

Analysis of COVID-19 patients' anamnestic and clinical characteristics yielded the following results: the average age of the patients was 30.3 ± 6.25 years, the average BMI value was 27.1 ± 4.6 kg/m^2, the average duration of the disease was 17.6 (6–34) days, and the length of hospital stay was 14.9 (4–30) days. Negative PCR test confirming the absence of SARS-CoV-2 virus in the oropharyngeal mucosa was obtained within 15.6 (6–31) days (on average). The major clinical manifestations observed in patients were as follows: loss of smell (34.9%), hyperthermia (33.3%), and cough (51.5%). Asymptomatic COVID-19 was observed in 15 patients (22.7%). Patients with mild, moderate and severe disease accounted for 25 (38%), 20 (30.2%) and 6 (9.1%) cases respectively.

All pregnant women received low molecular weight heparin and interferon alpha-2b. Antimicrobial therapy included amoxicillin–clavulanic acid combination (46%) and macrolides (28%). Carbapenems were considered as the drug of choice in severe cases. Supplemental oxygen was required in six cases (9%), of those in four cases (6%) it was used in the intensive care unit, and in two cases (3%) it was used during artificial ventilation. Corticosteroids (dexamethasone 12 mg/day for 3–4 days) and immunoglobulin therapy (0.5 g/kg for 3–4 days) were also prescribed. One maternal death from pulmonary embolism and multiple organ dysfunction syndrome progression associated with severe hematological disease occurred on day 33 after admission. Spontaneous abortion occurred at 21–22 weeks of gestation. Labor and prolongation of pregnancy occurred in 42 (63.6%) and 20 (30.3%) cases respectively, and spontaneous abortions occurred in four cases (6.1%) out of 66. Deliveries were performed in 42 women, of those eight cases of preterm labor (19%), and 34 cases of labor at term (81%). Caesarean section was performed in 17 patients (40.5%), and vacuum extraction vaginal delivery due to fetal distress was performed in two patients (4.8%); normal vaginal delivery occurred in 23 patients (54.7%). The average birth weight was 3,283 ± 477 g, and the average birth length was 52 ± 2.75 cm. The newborns were assigned Apgar score at one minute (7.8 ± 0.6) and five minutes (8.7 ± 0.5) of age. No cases of COVID-19 in newborn infants were registered. The infants were isolated from their mothers immediately after birth, until their

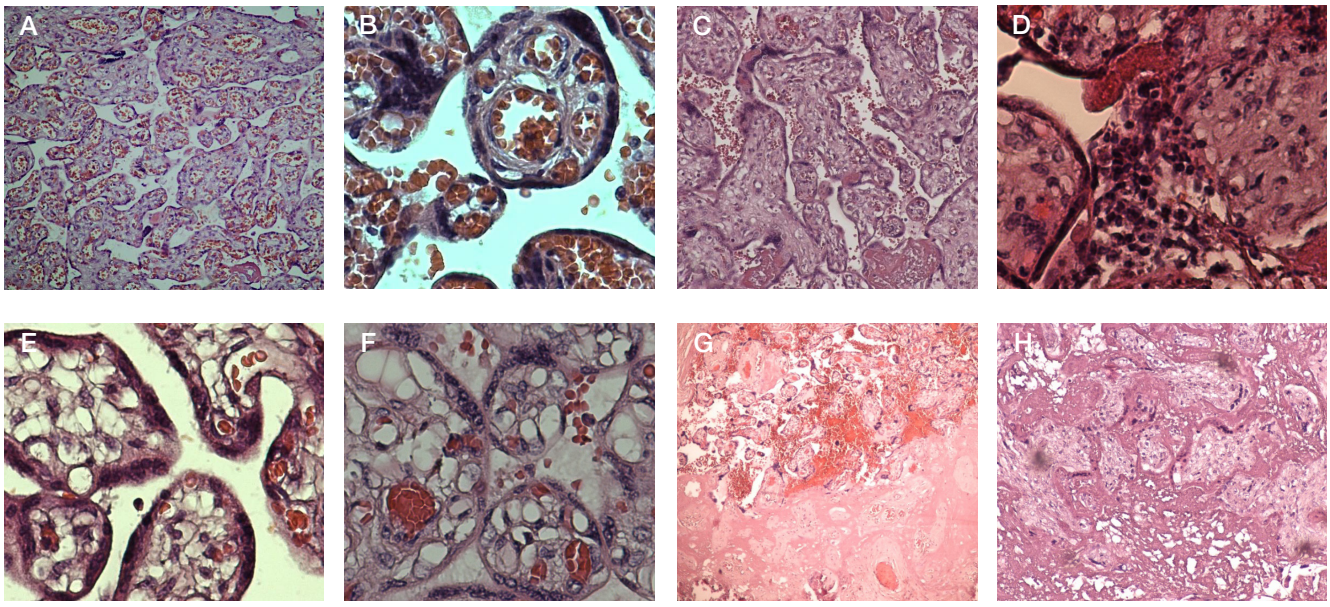

Fig. 1. Histological changes in placental villous trees of women with novel coronavirus infection. **A**. Placenta of women with uncomplicated pregnancies, one terminal villus contains 3–7 vessels; ×40. **B**. Placenta of women with uncomplicated pregnancies, one terminal villus contains 3–7 vessels 3–7; ×400. **C**. Placenta of patients with COVID-19; increased fibrinoid deposition; ×100. **D**. Placenta of patients with COVID-19; focal accumulations of inflammatory cells (macrophages, lymphocytes with a dash of plasmocytes and sporadic neutrophils); ×400. **E**. Placenta of patients with COVID-19; angiogenesis with predominant vascular branching: capillary number increase in one villus to more than 10 together with blood vessel diameter reduction; intact syncytiotrophoblast membranes attract attention. **F**. Placenta of patients with COVID-19; angiogenesis with predominant vascular branching: capillary number increase in one villus to more than 10 together with blood vessel diameter reduction. **G**. Placenta of patients with COVID-19; multiple infarction and hemorrhages in placental samples obtained from women with COVID-19. **H**. Placenta of patients with COVID-19

mothers tested negative twice for SARS-CoV-2. After getting a negative PCR test result the women resumed breastfeeding. All newborn infants were tested for SARS-CoV-2 immediately after birth, as well as at three and 10 days of age. All newborns tested negative for the virus. This could indicate lack of vertical transmission of the infection.

Placental tissue samples obtained from 42 patients who delivered in the red zone, one dead patient and 40 healthy women of the comparison group were sent to the Anatomical Pathology Department for further histological assessment and IHC analysis. Histological assessment revealed fewer than expected inflammatory conditions of placenta (umbilical cord, fetal membranes, and villous tree) in patients with COVID-19 (less than 10%). Focal forms of villitis prevailed (>3 affected fields of view out of 10 viewed at 200x magnification), which could be detected only when using IHC staining with antibodies to CD4 (marker of lymphocytes and macrophages) (Fig. 1A–F and 2A–D). In-depth analysis of complex placental changes in the index group patients revealed significant differences ($p < 0.05$), such as predominance of terminal villi hypercapillarization (moderate and severe degree) or pathological immaturity of the villous tree (see Fig. 1A–F). In patients with COVID-19, histological features of the villous tree corresponded to moderate and severe branching angiogenesis (angiogenesis with predominant vascular branching) (score 23 and 32, scales by Berniske, Kaufman, 2006). This indicated the compensatory mechanisms involvement and was more often characteristic of intrauterine hypoxia. The placental villous infarction rate was significantly higher in patients with novel coronavirus infection; it was mainly associated with severe forms of the disease ($p < 0.05$) (see Fig. 1A–F). The rate of intravillous hemorrhage and blood clots in the lumen of blood vessels showed the upward trend (see Fig. 1A–F). In the placenta of the woman with leukemia (the fatal case), up to 90% of the villous tree was affected, surrounded by infarcted fields, multiple hemorrhages and hematomas (subamniotic, intervillous, retrochorial), and massive fibrinoid deposition making it possible to distinguish only a few villi, some of which were dystrophic (see Fig. 1A–F). At the same time, in patients with COVID-19 who gave birth to healthy babies, the analysis of placental villous tree and the degree of villous syncytiotrophoblast damage revealed intact syncytiotrophoblast membranes, and no cells sloughed off the surface of the villi. In our opinion, the key consideration is the fact that in women with COVID-19 the trophoblast damage degree was less than 10%, i. e. it could be considered the normal value [7]; when entering the mother's bloodstream, the destroyed trophoblast compartments did not trigger maternal systemic inflammatory response observed in preeclampsia. During evaluation of IHC study results with the use of primary antibody to SARS-CoV-2 N-protein, lack of staining in most placental villi in women with mild forms of the disease drew the attention. In women with moderate and severe infection, the following features were detected: focal staining of individual syncytiotrophoblast villi membrane and cytoplasm, syncytial knots, small areas of the basement membrane, endothelium of individual villous vessels, and macrophages (Fig. 3A–F). No viral particles were detected in fetal membranes and umbilical cord. On the contrary, in patient with leukemia (the fatal case), individual villi positively stained with antibody to virus could be distinguished among the infracted fields and hemorrhages (Fig. 3; dark areas). Positively stained areas matched the villous syncytiotrophoblast contour; some villi with evident dystrophic changes were embedded in fibrinoid deposition (see Fig. 3A–F). Thus, the function of fibrinoid produced primarily of coagulated plasma proteins in the sites of villous tree damage is to separate maternal and fetal blood circulation in case of placental damage; when the viral particles enter the intervillous space, it seeks to localize and inactivate viral particles in order to prevent them from entering the fetal bloodstream. There were no signs of vasculitis and perivasculitis in blood vessels of patients with COVID-19 who gave birth to healthy babies, which was in contradiction to data on lung injury and damage to other organs resulting from granulomatous inflammation reported by a number of authors [8, 9]. In the index group,

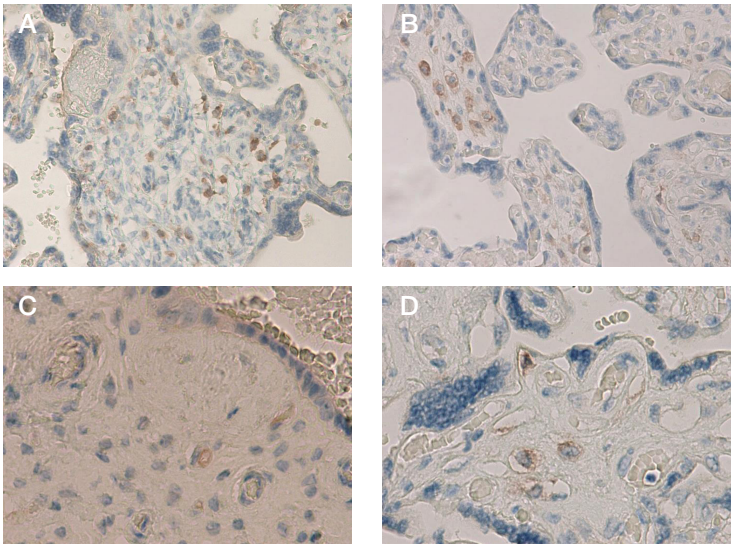

Fig. 2. CD4 expression in placental tissue in patients with COVID-19 and comparison group. **A**. Focal increase in the number of CD4+ macrophages and lymphocytes in the villous stroma in patients with COVID-19; ×200. **B**. Focal increase in the number of CD4+ macrophages and lymphocytes in the villous stroma in patients with COVID-19; ×200. **C**. Focal increase in the number of CD4+ macrophages and lymphocytes in the villous stroma in patients with COVID-19; ×400. **D**. Macrophages CD4+ in the villous stroma in women with uncomplicated pregnancy; ×400

IHC staining with primary antibody to TNFα revealed moderate staining of cytotrophoblast and syncytiotrophoblast membrane and cytoplasm, macrophage and lymphocyte cytoplasm, epithelium, as well as syncytial knots, functioning as the zones of trophiblast proliferation, extravillous cytotrophoblast and decidual cells. Prominent staining was observed in the perivillous fibrinoid (Fig. 4A–D). This demonstrated elevated plasma levels of proinflammatory markers compared to placenta. Assessment of IL8 revealed more prominent staining in the listed above structures, primarily in syncytiotrophoblast (Fig. 5A–F). As is known, syncytiotrophoblast forms the placental barrier, which functions as the main barrier for the virus. Downregulation of proinflammatory cytokine IL4 expression was observed in placenta of the index group patients with COVID-19 (Fig. 6A–D).

DISCUSSION

Thus, elevated proinflammatory cytokine synthesis and reduced levels of anti-inflammatory cytokines are observed in patients with novel coronavirus infection. The most prominent staining was detected in fibrinoid deposits. It should be noted that not only immune cells, but also placental structures are able to produce proinflammatory and anti-inflammatory cytokines, even when binding the innate immune receptors. Of particular note is the fact that even in case of 5–10 times increase in plasma levels of proinflammatory markers (cytokine storm), the placental levels of those were comparable with values characteristic of uncomplicated pregnancy. At the same time, upregulation of proinflammatory factors (TNFα, IL8) expression and downregulation of anti-inflammatory factors (IL4) were

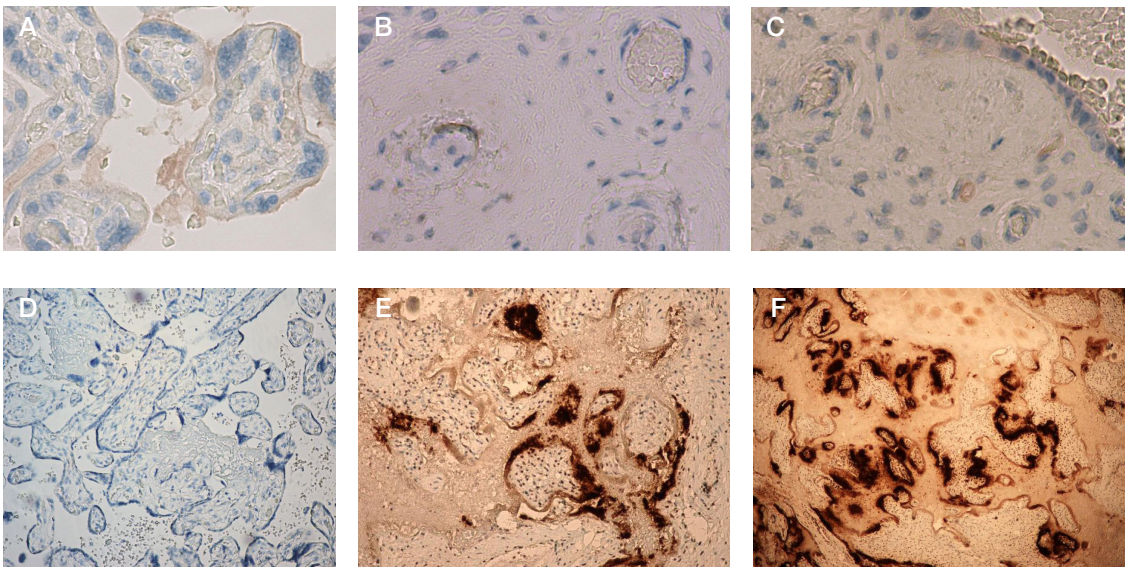

Fig. 3. Placental tissue immunohistochemical analysis using primary antibody to SARS-COV-2. **A**. Weak syncytiotrophoblast membrane and fibrinoid deposits staining in placental tissue of patients with COVID-19, intact syncytiotrophoblast membrane attracts attention; ×200. **B**. Weak focal staining of endothelium in individual villous vessels; ×400. **C**. Weak syncytiotrophoblast membrane and fibrinoid deposits staining in placental tissue of patients with COVID-19, intact syncytiotrophoblast membrane attracts attention, ×200; weak focal staining of endothelium in individual villous vessels, ×400. **D**. Negative staining. Placenta of women with no history of COVID-19; ×100. **E**. Prominent positive staining of viral particle accumulations around the perimeter of dystrophic villus. Placenta of a woman with acute leukemia, who died from COVID-19. The fetus died in utero from placental insufficiency at 20 weeks of gestation. Stained inflammatory cells are visible: lymphocytes, macrophages; ×100. **F**. Prominent positive staining of viral particle accumulations around the perimeter of dystrophic villus. Placenta of a woman with acute leukemia, who died from COVID-19. The fetus died in utero at 20 weeks of gestation from placental insufficiency; ×40

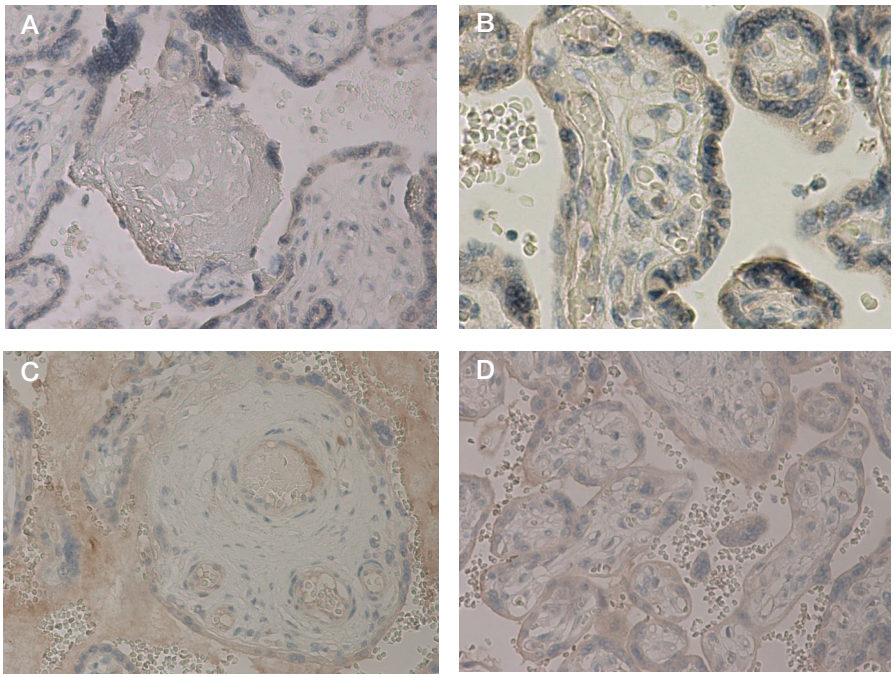

Fig. 4. TNFα expression in placental tissue in patients with COVID-19 and comparison group. **A**. TNFα expression in placental structures of women with uncomplicated pregnancy, weak staining of syncytiotrophoblast membrane and cytoplasm, as well as of vascular endothelium; no staining of fibrinoid deposits; ×100. **B**. TNFα expression in placental structures of women with uncomplicated pregnancy, weak staining of syncytiotrophoblast membrane and cytoplasm, as well as of vascular endothelium; no staining of fibrinoid deposits; ×200. **C**. TNFα expression in placental structures of women with COVID-19. Weak staining of vascular endothelium; positively stained fibrinoid deposits; ×200. **D**. TNFα expression in placental structures of women with COVID-19. Weak staining of vascular endothelium (Fig. C); as well as of syncytiotrophoblast membrane and cytoplasm, and syncytial knots (Fig. D); positively stained fibrinoid deposits (Fig. C); ×200

observed in placental structures of patients with moderate and severe COVID-19.

An important reason for impairment of umbilical blood flow is blood clot and sludge formation in the umbilical cord and chorionic blood vessels, also referred to as fetal thrombotic vasculopathy [10, 11]. Hypercapillarization of terminal villi is a compensatory mechanism for hypoxia, which is attributable to elevated level of angiogenic factors promoting growth of new blood vessels, such as VEGF. In case of prominent terminal villi hypercapillarization (32–33 points in accordance with the scale) [12] rearrangement occurs during angiogenesis resulting in slowing down in microcirculation and fetoplacental blood flow. Therefore, platelet aggregation in the capillary lumen increases, and microthrombi are formed. Upregulation of these processes

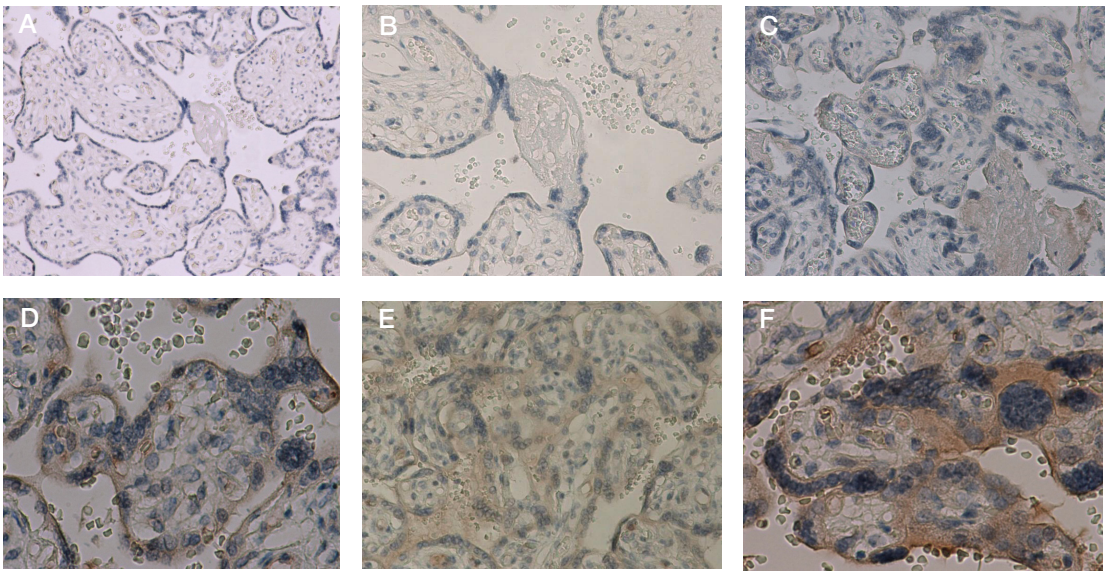

Fig. 5. IL8 expression in placental tissue in patients with COVID-19 and comparison group. **A**. IL8 expression in placental structures of women with uncomplicated pregnancy, weak staining of syncytiotrophoblast membrane and cytoplasm, as well as of vascular endothelium; no staining of fibrinoid deposits; ×100. **B**. IL8 expression in placental structures of women with uncomplicated pregnancy, weak staining of syncytiotrophoblast membrane and cytoplasm, as well as of vascular endothelium; no staining of fibrinoid deposits; ×200. **C**. IL8 expression in placental structures of women with COVID-19; IL8 expression in patients with mild forms of the disease: weak staining predominantly in the syncytiotrophoblast membrane and cytoplasm; weak staining of fibrinoid deposits; ×100. **D**. IL8 expression in placental structures of women with COVID-19; IL8 expression in patients with moderate forms of the disease: more prominent staining of placental structures, mostly in the syncytiotrophoblast; intensively stained fibrinoid deposits; some villi are embedded in fibrinoid deposits; ×400. **E**. IL8 expression in placental structures of women with COVID-19; IL8 expression in patients with moderate forms of the disease: more prominent staining of placental structures, mostly in the syncytiotrophoblast; intensively stained fibrinoid deposits; some villi are embedded in fibrinoid deposits; ×200. **F**. IL8 expression in patients with moderate forms of the disease: more prominent staining of placental structures, mostly in the syncytiotrophoblast; intensively stained fibrinoid deposits; some villi are embedded in fibrinoid deposits

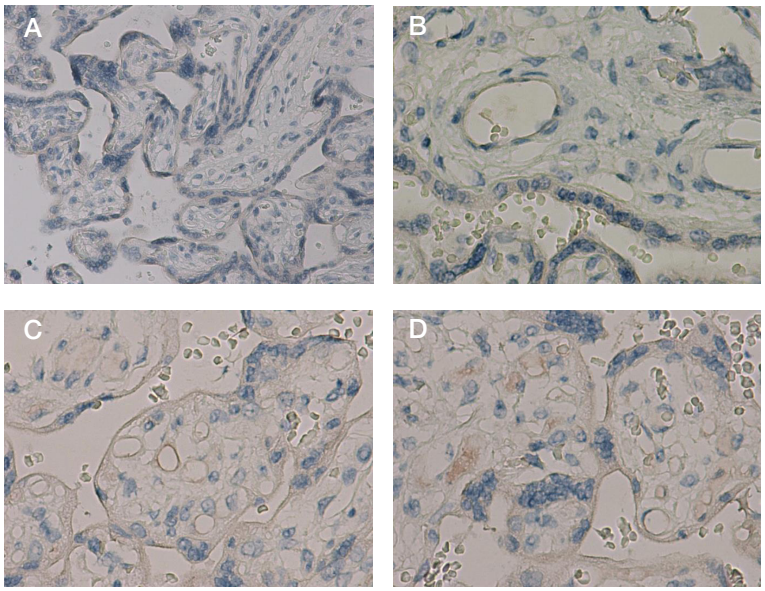

Fig. 6. Expression of proinflammatory mediator IL4 in placental villi in patients with COVID-19 and comparison group. **A**. IL4 expression in placental structures of women with uncomplicated pregnancy; weak staining of syncytiotrophoblast and villous vessel endothelium; ×100. **B**. IL4 expression in placental structures of women with uncomplicated pregnancy; weak staining of syncytiotrophoblast and villous vessel endothelium; ×400. **C**. IL4 expression in placental structures of women with COVID-19: decreased staining is visible. **D**. IL4 expression in placental structures of women with COVID-19: decreased staining is visible

results in focal villous stromal fibrosis; changes initially focused on hypoxia negative impact compensation facilitate gradual transition to decompensation. In our study, the frequency of moderate and severe terminal membrane hypercapillarization in the index group was significantly higher than in comparison group. The study results showed higher proportion of infarctions, as well as the presence of chorionic vessels thrombosis, and intervillous and subamniotic hematomas in the index group compared to controls. In addition to mechanical factors, blood clot formation is caused by violation of blood rheological properties (slowdown in blood flow, decreased diameter of blood vessels), and coagulation system disorders. These factors are characteristic of novel coronavirus infection. Morphological features of placenta suggested that acute fetal hypoxia during labor occurred in cases of chronic fetal hypoxia in the mother-placenta-fetus system or any other disturbing factor influence during pregnancy [13, 14]. As a result, the villous tree rearrangement occurred, and the subclinical placental insufficiency developed. The influence of additional triggers during labor (uterine contractile activity, umbilical cord compression, etc.) resulted in decompensation with clinical manifestations of acute fetal hypoxia.

Evaluation of IHC analysis results revealed no significant increase in the proinflammatory marker levels against the background of decreased inti-inflammatory marker levels in patients with moderate and severe infection.

CONCLUSIONS

In the recent decade, advances in immunology and broader understanding of innate and acquired immunity mechanism resulted in growing interest in studying the individual differences of immune response associated with infectious inflammatory diseases. Studying the immune response differences in individuals with predominant Th1 or Th2 response, which largely determines the clinical, morphological and immunological features of the inflammatory response, is the most productive. Thus, pregnancy may be considered as a factor contributing to more favorable course of COVID-19. The reported higher proportion of hypoxia during labor may be associated with coagulation system disorders and result from higher proportion of chorionic blood vessel thromboses, as well as from intervillous, subamniotic, retrochorial hematomas, and villous tree infarctions. Placenta functions as an “airbag” for the fetus, and the intact placental barrier protects the fetus against COVID-19. However, under certain conditions, in case of “broken” placental barrier the novel coronavirus infection may be dangerous for the fetus: the infection can induce the cytokine-storm-type changes. Placenta minimizes the burden on the fetus. The vast majority of babies are born healthy, and the placental levels of proinflammatory cytokines are comparable with those in comparison group. Intact placenta and placental barrier protect the unborn baby against infections, and the impact on the child’s development is minimal. Taking into account higher proportion of hypoxia occurring during labor in infants born from mothers with COVID-19, the obstetrical tactics for mothers with COVID-19 should be decided individually based on the risk factors; continuous cardiotocography should be used during labor. It may be appropriate to conduct IHC analysis of placenta in puerperant women with suspected novel coronavirus infection COVID-19 using primary antibodies to Sars-CoV-2 in order to fine-tune the tactics of neonatal management and to predict possible neonatal complications.

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SONOGRAPHIC FEATURES OF OVARIAN TUMORS IN PREGNANT WOMEN BEFORE SURGICAL INTERVENTION AND CHEMOTHERAPY

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The problem of ovarian tumor differential diagnosis is solved using comprehensive ultrasound examination at different levels of efficiency. However, ultrasound imaging is an operator-dependent technique due to subjective interpretation of results. The study was aimed to assess the ultrasound signs of benign and malignant ovarian germ cell tumors (OGCTs) in pregnant women in order to determine the feasibility of surgical treatment and chemotherapy. The study was carried out using the logistic regression models. A group of 199 pregnant women with benign and malignant OGCTs were enrolled. Of them, 183 patients had benign, and nine patients had malignant tumors. In order to assess menstrual function and fertility, seven pregnant women were enrolled, who had previously received treatment for malignant OGCTs. Pre-operative assessment results were compared with morphological assessment data. Organ-preserving surgical treatment was performed (unilateral adnexectomy); if necessary, the patients received cisplatin-based chemotherapy. Perinatal outcomes were assessed. The median observation time between the malignant OGCT detection and the end of the study was 66 months (12–240 months). It was found that comprehensive ultrasound examination and logistic regression models (sensitivity 100%, specificity 92.3%, overall accuracy 92.8%) enabled differential diagnosis of benign and malignant OGCTs. The number of unnecessary surgical procedures in patients with benign OGCTs was been limited, the pregnancy and childbirth outcomes were improved. Nine pregnant women received organ-preserving surgical treatment for malignant OGCTs, and three patients received chemotherapy after surgery, which allowed the patients to realize their reproductive potential.

Keywords: ultrasound examination, morphological assessment, ovarian tumors, pregnancy

Author contribution: the authors contributed to study management and manuscript writing equally, read and approved the final version of the article prior to publishing.

Compliance with ethical standards: the study was approved by the Ethics Committee of Pirogov Russian National Research Medical University (protocol № 176 dated June 25, 2018). The informed consent was submitted by all study participants.

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УЗ-ПРИЗНАКИ ГЕРМИНОГЕННЫХ ОПУХОЛЕЙ ЯИЧНИКОВ У БЕРЕМЕННЫХ ПЕРЕД ХИРУРГИЧЕСКИМ ЛЕЧЕНИЕМ ИЛИ ХИМИОТЕРАПИЕЙ

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Проблему дифференциальной диагностики опухолей яичников решают с помощью комплексного ультразвукового исследования (УЗИ), несмотря на то что оно является операторозависимым методом из-за субъективной интерпретации полученных данных. Целью исследования было оценить УЗ-признаки доброкачественных и злокачественных герминогенных опухолей яичников (ДГОЯ и ЗГОЯ) у беременных для определения возможности хирургического лечения и проведения химиотерапии (ХТ). Исследование проводили с помощью логрегрессионных моделей. В нем участвовали 199 беременных женщин с ДГОЯ и ЗГОЯ. Из них 183 пациентки имели доброкачественные опухоли, девять — злокачественные. Для оценки менструальной функции и фертильности в исследование были включены семь беременных после проведенного ранее лечения ЗГОЯ. Полученные на дооперационном этапе результаты обследования сопоставляли с морфологическими исследованиями. Выполняли хирургическое лечение в органосохраняющем объеме (одностороннюю аднексэктомию), при необходимости — ХТ на основе цисплатина. Изучали перинатальные исходы. Медиана наблюдения с момента выявления ЗГОЯ до окончания исследования составила 66 месяцев (12–240 месяцев). Обнаружено, что дифференцировать ДГОЯ со ЗГОЯ возможно с помощью комплексного УЗИ и логрегрессионных моделей (чувствительность метода — 100%, специфичность — 92,3%, суммарная точность — 92,8%). Ограничено число нецелесообразных операций при ДГОЯ и улучшены исходы беременности, родов. Хирургическое лечение ЗГОЯ у девяти беременных выполнено в органосохраняющем объеме, у троих — с последующей ХТ, что позволило реализовать репродуктивный потенциал.

Ключевые слова: ультразвуковое исследование, морфологическое исследование, опухоли яичников, беременность

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Differential diagnosis of ovarian tumors in pregnant women currently remains one of the unsolved problems in obstetrics. According to some authors, ovarian tumors and tumor-like formations are detected, on average, in 2–3% of pregnant women; the malignant ovarian tumors detection rate in pregnant women is 1 per 10,000–50,000 patients [1–6].

Ovarian germ cell tumors (OGCTs) account for 20–30% of all ovarian tumors; 2–5% of OGCTs are malignant [7, 8]. The most common benign tumor is mature teratoma. The incidence of mature teratoma reaches 12% of all ovarian tumors. Rare teratomas include struma ovarii (1–2.7% of all ovarian teratomas). According to literary sources, in

5–20% of cases struma ovarii can transform into carcinoma [9–12]. Malignant germ cell tumors (malignant OGCTs) are divided into two groups based on the clinical and histological features: dysgerminomas and non-dysgerminomas (yolk sac tumor, embryonal carcinoma, choriocarcinoma, immature teratoma, polyembryoma, and combinations of all listed types). Dysgerminomas account for up to 50% of all malignant OGCTs, this morphological type has more favorable prognosis compared to non-dysgerminoma [7, 13–16].

Currently, the problem of ovarian tumors differential diagnosis is solved using comprehensive ultrasound examination at different levels of efficiency. However, ultrasound imaging is an operator-dependent technique due to subjective interpretation of results. Therefore, to standardize the ultrasound imaging results, improve the ovarian tumor diagnosis accuracy and stratify the risk of malignant OGCT in order to determine the strategy of the further patients' management and treatment, the algorithms and complex predictive models have been constructed (RMI, IOTA (simple rules, LR1, LR2), ROMA, Kentucky morphology index (MI), O-RADS). According to ESGO guidelines (2017), in case of initial stage malignant OGCT detection, peritoneal surgical staging, and surgical removal of affected adnexa with preservation of contralateral ovary and pregnancy are recommended. However, biopsy of intact ovary and retroperitoneal lymph node dissection are considered non-viable options [17]. Cytoreductive surgery with maximum removal of tumor nodes and preservation of contralateral ovary without total lymphadenectomy is indicated to patients with advanced stage tumors. Chemotherapy is not recommended to patients with stage IA immature teratoma G1 or stage IA dysgerminoma. Chemotherapy is required in patients with advanced stage tumors. The platinum etoposide combination is considered the standard regimen (BEP or EP) [6, 18]. Organ-preserving surgery makes it possible to preserve reproductive function and is in line with treatment concept for improved quality of life. In most cases, menstrual function is restored after chemotherapy, therefore, normal pregnancy might be expected [16, 19].

The study was aimed to assess the ultrasound signs of benign and malignant OGCTs in pregnant women in order to determine the feasibility of surgical treatment and chemotherapy.

METHODS

In 2000–2020 a study was carried out involving 199 patients with histologically verified OGCTs. Inclusion criteria: consent to participate in the study; pregnancy; ultrasound findings confirming the pregravid or gravid ovarian tumor, subsequent surgical treatment and morphological verification of OGCT. Exclusion criteria: the pregnant woman's refuse to participate in the study; threatened miscarriage; intrauterine infection; defects in a fetus diagnosed before the study.

Ultrasonographic examination was performed with the Voluson E8 system (General Electric; USA) combined with transabdominal and transvaginal Doppler color flow mapping and pulse wave Doppler measurements. The tumor ultrasound features were assessed using the earlier proposed models [20].

In 192 pregnant women, the logistic regression models were used to assess the ultrasound signs of benign and malignant OGCTs, as well as to determine the feasibility of surgical treatment and chemotherapy. Of them, a group of 183 patients (95.3%) had benign tumors: 180 patients had mature teratomas, and three patients had struma ovarii (Table 1). Among them, 32 patients (17.5%) underwent surgery during pregnancy, 117 patients (63.9%) received surgical treatment during a cesarean section, and 34 patients (18.6%) received it postnatally: 11 patients (6%) 3–5 days, and 23 patients (12.6%) 3–6 months after giving birth. In most cases patients with small-sized benign OGCTs underwent resection of the ovaries, and patients with tumors larger than 10 cm in diameter underwent unilateral adnexectomy. In nine patients (4.7%), malignant OGCTs were detected during progressive pregnancy. Seven patients (77.8%) out of nine had stage I tumors, and two patients had stage III tumors. Pregnancy was preserved in all monitored patients. Six patients out of nine underwent surgery during different stages of gestation. Surgical treatment of three patients included the following procedures: cesarean section and adnexectomy with surgical staging. In four cases the tumor morphology was characteristic of pure dysgerminoma, and in one case the tumor was a combination of dysgerminoma with yolk sac tumor. Three pregnant women had immature teratomas, and one woman had a combination of immature teratoma with yolk sac tumor. Among nine patients with tumor process detected during pregnancy, three patients received drug treatment after surgery. Two patients received first-line BEP chemotherapy after giving birth because of stage III disease or yolk sac tumor, and one patient received two cycles of adjuvant carboplatin-based chemotherapy starting from the 18th week of gestation. In order to access menstrual function and fertility, medical records together with pregnancy and childbirth outcomes were studied in seven patients, who had received treatment for malignant OGCTs before pregnancy. Six out of seven patients have stage I tumors, and one patient had stage III tumor. All patients underwent organ-

Table 1. Distribution of pregnant women based of tumor morphology and stage

Morphological type	FIGO-TNM stage	Abs.	Total
Dysgerminoma	T1aN0M0/IA	5	9
	T1bN0M0/IB	1	
	T1cN0M0/IC	2	
	T3N1M0/IIIC	1	
Yolk sac tumor	T1cN0M0/IC	1	1
Immature teratoma	T1aN0M0/IA	2	4
	T1cN0M0/IC	1	
	T3cN0M0/IIIC	1	
Mixed type germ cell tumor	T1aN0M0/IA	1	2
	T3N1M0/IIIC	1	
Total		16	16

Table 2. OGCT surgical treatment extent

Surgical treatment extent	Benign OGCTs	Malignant OGCTs	
		Primary	Reintervention
Resection of the ovary	134	2	
Bilateral resection of the ovaries	19		
Biopsy of the second ovary + omentectomy			1
Unilateral adnexectomy	20	5	
Unilateral adnexectomy + resection of the second ovary	10	1	
Unilateral adnexectomy + biopsy of the second ovary + omentectomy		3	
Unilateral adnexectomy + resection of the second ovary + omentectomy + multiple peritoneal biopsy		2	
Unilateral adnexectomy + resection of the second ovary + omentectomy + multiple peritoneal biopsy + retroperitoneal lymph node biopsy		2	2
Unilateral adnexectomy + resection of the second ovary + omentectomy + multiple peritoneal biopsy + retroperitoneal lymph node dissection		1	1
Retroperitoneal lymph node dissection, peritoneal tumor nodes removal			1

preserving surgical treatment (adnexectomy). In five cases, the tumor morphology was characteristic of pure dysgerminoma; one patient had immature teratoma, and another one had a yolk sac tumor. Six out of seven patients received BEP/E chemotherapy: one patient with immature teratoma G2, one patient with a yolk sac tumor, and four patients with IC stage or later stage dysgerminoma.

The surgical treatment extent in patients with malignant OGCTs was affected by each particular situation and by the urgent histological examination results (Table 2).

Histological examination was performed by standard methods. The morphological diagnoses were provided in accordance with the WHO Classification of Tumors of Female Reproductive Organs issued in 2014 [21].

Statistical analysis was carried out using the SPSS 15 software package (IBM; USA). The differences were considered significant when $p < 0.05$.

RESULTS

The study showed that in pregnant women the scanograms of mature teratomas were characterized by polymorphous echographic pattern. In most cases mature teratomas were unilateral; bilateral lesion was identified in 29 patients (15.8%). The tumor diameter varied significantly in the range of 0.5–15.0 cm, and the small-sized tumors (0.5–3.0 cm) were detected in 38 cases (21%).

Based on the study results, in 75 cases (41%), the ultrasound semiotics of benign OGCTs included mixed structure with predominant cystic component. In 49 patients (27%), solid component prevailed over cystic component (cystic-solid structure). In 33 cases (18%), neoplasms were represented by solid component with clearly visible capsule. The tumors were completely anechogenic (cystic variant) only in 26 cases (14%).

The color and energy Doppler mapping revealed either single colored peripheral blood flow loci with resistance index (RI) of 0.4–0.6, or tumor avascularity. When assessing tumor vascularization using color and energy Doppler mapping, blood flow with peripheral zones of vascularization was detected in 53 patients (29%) with benign OGCTs.

The proposed logistic regression models for differential diagnosis of benign, borderline and malignant ovarian tumors in pregnant women possessed high reproducibility, sensitivity and specificity.

When diagnosing teratomas in pregnant women (Table 3) using the model, the accuracy exceeded 90%, and the model sensitivity and specificity were 97% and 95%, respectively (Fig. 1).

All tumors were characterized by mixed echogenicity.

Based on the study results, the ultrasound semiotics of malignant OGCTs was represented by solid masses found in 5 patients (55.6%). Cystic and solid malignant OGCTs were detected in three cases (33.3%), and the mixed structure with predominant cystic component was revealed in one case (11.1%). In one case, the solid tumor component of higher echogenicity was represented by parenchymal papilla of more than 10 cm in diameter, and amounted to more than 80% of the total tumor volume. Hypervascularization was detected in 100% of cases. Ascitis was diagnosed in 3 patients (33.3%).

The distinctive ultrasound signs of dysgerminoma were as follows: solid (lobulated) tumor, clear margins and rough external contour, medium-level echogenicity and multiple centrally located blood flow loci. In all cases, in scanograms of pregnant women, immature teratomas were represented by hypervascular cystic and solid masses. On the ultrasound scan the yolk sac tumor, 12 cm in diameter, was represented by a solid mass with multiple cystic cavities of various size, which contained suspension. Color Doppler mapping revealed multiple loci of blood flow with low RI values. In cases of mixed germ cell tumors (dysgerminoma + yolk sac tumor, immature teratoma + yolk sac tumor) the obtained ultrasound features and Doppler measures were consistent with ultrasound signs characteristic of malignant neoplasm in 100% of patients (unclear, uneven contour, solid component amounting to 80–100% of tumor volume, hypervascularization together with high values

Table 3. Results and regression coefficients

Histotype	Model accuracy	Area under the ROC curve	Parameters	Regression coefficients
Mature teratoma	0.99	0.994	Constant	–171.05
			PI	14.81
			Localization	14.65
			Internal contours	36.33

of blood flow velocity and low values of RI, presence of ascitis).

When constructing the model for differential diagnosis of benign, borderline and malignant ovarian tumors, the Spearman's rank correlation coefficients were calculated and included in the regression model for diagnosis of borderline and malignant ovarian tumors in pregnant women (Table 4).

When diagnosing borderline and malignant ovarian tumors in pregnant women, the model sensitivity was 100%, the specificity was 92.3%, and the overall accuracy was 92.8% (Fig. 2).

Surgical treatment of patients with benign and malignant OGCTs was performed after tumor detection (see Table 2). In the group of patients with benign OGCTs, who underwent surgery during different stages of gestation (n = 32), the emergencies in the first–second trimesters (adnexal torsion resulting from the tumor) were considered indications in four cases (12.5%); tumor size and bilateral tumor in the second trimester determined the kind of surgical treatment in 28 cases (87.5%). In 13 patients (11.1%) the presence of neoplasm was considered an indication to surgery. The other 104 pregnant women (about 89%) underwent surgery because of the combination of indications (uterine scar, placenta accreta, preeclampsia, breech presentation, hemolytic disease of the fetus, narrow pelvis, fetal malposition, and the presence of a tumor). In the group of 106 patients (90.5%) with benign OGCTs the delivery was performed at term. Preterm operative vaginal delivery due to life-threatening birth defects, placental abruption, preeclampsia not amenable to therapy, and premature discharge of amniotic fluid was performed in 11 cases (9.4%) between 25–35 weeks of gestation. All infants were born alive; the newborns' morphofunctional characteristics corresponded to their gestational age (full-term infants with Apgar score 7–9 weighted 2,500–4,150 g; premature infants with Apgar score 3–9 and Silverman Andersen respiratory severity score 3–5 weighted 650–2,850 g). Surgical treatment in the form of resection of the ovaries was performed postpartum in patients with small-sized tumors.

The median age of nine patients at the moment of malignant OGCT detection was 26 years (20; 32).

Three patients out of nine with malignant OGCTs received drug treatment. Two patients received BEP as first-line chemotherapy after delivery. In cases of stage IA dysgerminomas only surgical treatment was performed. Combined-modality therapy (surgery + chemotherapy) was used in patients with stage III immature teratomas (G2) and mixed germ cell tumors (stages I and III). It should be noted that one of these patients received two cycles of adjuvant carboplatin-based chemotherapy during pregnancy, starting from the 18th week. The mixed ovarian germ cell tumor (combination of immature teratoma with yolk sac tumor) was diagnosed at 12–13 weeks of gestation; surgery was performed 2 days later. Surgical treatment included unilateral adnexectomy, biopsy of the contralateral ovary, omentectomy, multiple peritoneal biopsy, and peritoneal washing.

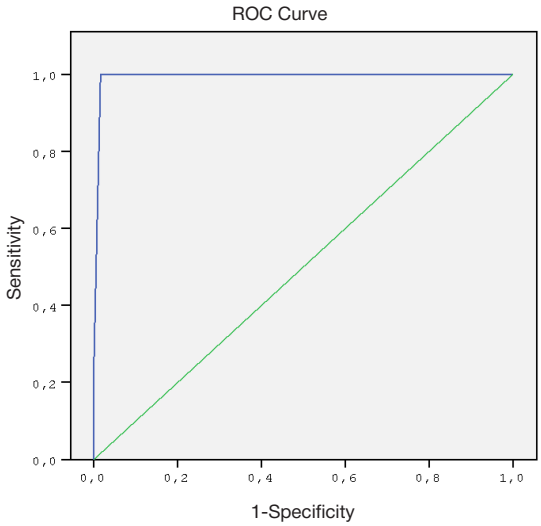

Fig. 1. ROC curve for the model of mature teratoma identification

No fetal injury was detected after the combined-modality therapy. This full-term pregnancy ended in second livebirth. The full-term newborn girl weighted 3,850 g, and was 55 cm long. She was assigned the Apgar score 8–9. The patient is under observation with no signs of disease progression. The child grows and develops normally, matching her age.

Recurrent tumor was detected in one patient, who had previously undergone surgery because of malignant OGCT at 25 weeks of gestation. Surgical treatment included unilateral adnexectomy; histologic diagnosis of mixed germ cell tumor (combination of dysgerminoma with yolk sac tumor) was established. Preterm labor occurred at 30 weeks of gestation: the preterm cesarean section was performed together with surgical removal of anterior abdominal wall tumor and ovarian ligament stump, resection of the contralateral ovary, omentectomy, paracolic gutters biopsy, surgical removal of paraaortic lymph node (based on the histological assessment results, the mixed germ cell tumor with predominant yolk sac tumor structures was diagnosed). The newborn boy weighed 1420 g and was 40 cm long. He was assigned Apgar score 6, and Silverman Andersen respiratory severity score 4.

Among nine pregnant women with malignant OGCTs detected during pregnancy, six women (66.6%) underwent cesarean section (four women because of tumors, one woman because of postterm pregnancy, and one woman because of preterm labor); three patients (33.3%) delivered per vias naturalis.

The median observation time in patients with malignant OGCTs was 66 months (12–240 months), one patient with stage III mixed germ cell tumor (combination of dysgerminoma and yolk sac tumor) died.

The further combined-modality therapy (surgery +

Table 4. Spearman's rank correlation coefficients included in the regression model for diagnosis of borderline and malignant ovarian tumors in pregnant women

Predictor variable	Ovarian tissue	RI	PI	Localization of blood vessels
Ovarian tissue	–	–	–	–
RI	r = –0.145	–	–	–
	p = 0.01			
PI	r = 0.326	r = 0.097	–	–
	p = 0.046	p = 0.01		
Localization of blood vessels	r = 0.059	r = 0.773	r = 0.294	–
	p = 0.01	p = 0.01	p = 0.01	

chemotherapy) outcome monitoring in six out of seven patients with malignant OGCTs, who had received treatment before pregnancy, revealed no menstrual function disorders. The median time between the end of chemotherapy and the end of the study was 150 months (48–216 months). The first pregnancy occurred in 1–3 years (two patients), 4–6 years (two patients), and 7–10 years (one patient) after chemotherapy. Only one patient became pregnant after 12 years with the help of IVF. A total of 11 pregnancies occurred after the malignant OGCT treatment. All pregnancies ended in birth of healthy babies. During the follow-up period four patients became pregnant and delivered second time. No pregnancy and labor complications were observed in these patients.

In five patients (71%), who had received treatment for malignant OGCTs before pregnancy, spontaneous labor at term was observed. In two cases (29%) cesarean section was performed because of uterine scar and macrosomic postterm delivery. The newborns' condition matched their gestational age, they were assigned Apgar score 8–9.

DISCUSSION

OGCTs account for 20–30% of ovarian tumors. According to literary sources, these tumors are characterized by the greatest structural diversity. Mature teratomas account for 12% of all ovarian tumors and for up to 97% of all OGCTs [8, 22–24]. Seven types of benign and several types of malignant neoplasms have been reported [22]. In 2005, three major types of ultrasound mature teratoma appearance were distinguished: cystic type (47–60%), predominance of solid component (20–43%), and mixed structure (9–20%) [24]. A rare type of teratoma is struma ovarii, which accounts for 1–2.7% of all ovarian teratomas. In 75% of cases, ovarian strumae have a specific feature: the spongy texture area of medium-level echogenicity with single hyperechoic inclusions inside the tumor [23].

There is evidence that the ultrasound imaging sensitivity when examining teratomas is 92.3%, and the method specificity is 99.4%; when diagnosing borderline and malignant ovarian tumors in pregnant women, sensitivity and specificity are 88.9 and 98.2% respectively [25]. In our study, with the use of contemporary ultrasound imaging techniques for differential diagnosis of OGCTs, we managed to establish the correct diagnosis during the pre-operative assessment of patients in almost all cases despite the tumor structure variability. It can be concluded that tumors with increased risk of malignant transformation are characterized by solid or mixed structure with predominant solid component, as well as by hypervascularization.

Ovarian tumors contribute to pregnancy complications, as well as to high maternal and fetal morbidity. The most common obstetric complication is miscarriage (its frequency is 30–75%); miscarriage may result from underlying process, surgical treatment and chemotherapy [26–28]. According to literary sources, the incidence of ovarian tumor torsion is 6–9%, the incidence of tumor capsule rupture is 12–14.7%, and the incidence of birth canal obstructed by the tumor is 14–21% [25]. In our study, the threatened miscarriage rate was 18.2%, adnexal torsion was detected in 2.1% of patients, hematoma with abscess formation was a complication of one pregnancy, and the birth canal obstruction rate reached 8.9%. Two pregnancies ended in preterm labor 3 and 5 weeks after surgical treatment of malignant OGCTs.

Unilateral adnexectomy is the optimal primary surgical treatment regardless of the tumor stage. According to world's literature, in case of malignant OGCT diagnosed during

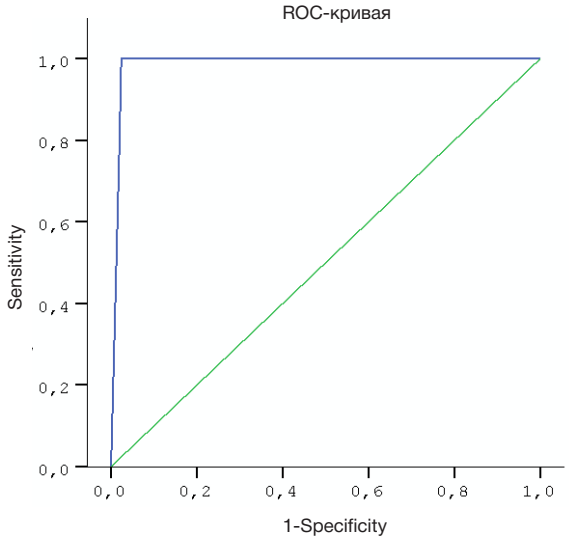

Fig. 2. ROC curve for the model of borderline and malignant ovarian tumor diagnosis in pregnant women

pregnancy, the treatment tactics are focused on prolongation of pregnancy with combined-modality therapy: surgery in the second trimester with subsequent chemotherapy or follow-up [4, 16, 29]. During our study, pregnancy was preserved in all patients, and all surgical procedures were organ-preserving (unilateral adnexectomy), which was in line with international guidelines. In a number of studies, 10 pregnant patients were monitored. Four pregnancies ended in abortions; six patients underwent adnexectomy at different stages of gestation [16, 30]. Currently, owing to high sensitivity of malignant OGCTs to chemotherapy, as well as to possibility to cure up to 80% of patients even with advanced stage tumors, the surgical intervention extent has become possible allowing us to preserve the patient's reproductive function [16]. According to literature, chemotherapy is not indicated to patients with malignant OGCTs after surgery in case of stage IA immature teratoma GI or stage IA dysgerminoma. Chemotherapy is indicated to patients with advanced stage tumors. BEP or EP is the standard chemotherapy regimen. Three out of nine pregnant women with malignant OGCTs received BEP chemotherapy as first-line therapy after surgery: two patients received chemotherapy after giving birth, and one patient received it during pregnancy, starting from the 18th week of gestation (two cycles of adjuvant carboplatin-based chemotherapy).

It can be assumed that the most aggressive malignant neoplasms are found during pregnancy. Therefore, in case of stage II–III non-dysgerminoma detection during pregnancy, chemotherapy should be prescribed after primary surgical treatment and the implantation and organogenesis completion, without waiting for the baby to be born. Exposure to bleomycin, etoposide and cisplatin in the second and third trimesters is safe for the fetus [4]. In patients with malignant OGCTs who had received pregravid organ-preserving surgery and drug treatment, pregnancies occurred and progressed, and healthy babies were born. In patients with benign OGCTs, the absence of ultrasound signs of malignant transformation made it possible to avoid surgery and related perinatal complications during pregnancy, and to perform surgical treatment of tumors postpartum.

CONCLUSIONS

Comprehensive diagnosis of OGCTs, which comprised determining the ultrasound criteria of malignancy, made it

possible to provide rational management of pregnancy and childbirth, surgical treatment and chemotherapy of malignant OGCTs, as well as to preserve patients' health and reproductive function.

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PLANNING ORTHODONTIC FRONTAL TEETH INCLINATION AND ESTIMATING BONE THICKNESS FROM CONE-BEAM COMPUTED TOMOGRAPHY IMAGES

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Changing the vestibulo-oral inclination (torque) of the frontal teeth is an important component of orthodontic treatment. Cone-beam computed tomography is recommended as an accurate diagnostic tool allowing the orthodontist to estimate the safety of frontal tooth movement and inclination. This tool is helpful in measuring bone thickness at different levels of the tooth root and estimating incisal inclination and position. The aim of our study was to analyze bone thickness in patients with pathologically and normally inclined teeth using CT images and to create a universal table that will provide useful information about the thickness of the bone around different segment of the root required for a safe change in tooth inclination. Using the proposed table, the orthodontist can assess the feasibility of the planned tooth movement in the setting of changed tooth inclination, with due account of critical bone deficit regions. This will ensure the safety of tooth movement, stable retention and a positive treatment outcome.

Keywords: orthodontic treatment, bone thickness, CBCT, center of resistance, torque

Author contribution: Kopetskiy IS analyzed the literature; Meskhiya NG conducted the study, gathered and analyzed clinical data; Kopetskaya AI processed and analyzed source medical records; Eremin DA, Orekhova DD performed data analysis.

Compliance with ethical standards: the study was approved by the Ethics Committee of Piragov Russian National Research Medical University (Protocol No.116 dated March 26, 2012); informed consent was obtained from all study participants or their legal representatives.

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ИЗМЕНЕНИЕ ТОРКА ФРОНТАЛЬНЫХ ЗУБОВ С ОЦЕНКОЙ ТОЛЩИНЫ КОСТНОЙ ТКАНИ НА КОНУСНО-ЛУЧЕВОМ КОМПЬЮТЕРНОМ ТОМОГРАФЕ

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Изменения вестибуло-орального положения фронтальных зубов (торка) — важный этап в ортодонтическом лечении. Для наиболее точной диагностики, обеспечивающей врачу-ортодонту безопасное перемещение и изменение наклона фронтальной группы зубов, рекомендовано использовать конусно-лучевую компьютерную томографию. Данная методика позволяет оценить толщину костной ткани на различных уровнях корня исследуемого зуба, измерить длину корней фронтальных зубов, определить наклон и положение резцов. Целью исследования было с помощью компьютерных томограмм пациентов с патологическими и физиологическими наклонами зубов провести анализ толщины костной ткани и разработать универсальную таблицу, использование которой даст врачу-ортодонту сведения о необходимой толщине костной ткани в различных сегментах корня при изменении вестибуло-орального наклона. Используя данную таблицу, врач может оценить возможность запланированного перемещения зуба при изменении торка, приняв во внимание зоны критического дефицита кости, что обеспечит безопасное перемещения зуба, стабильную ретенцию и благоприятный исход ортодонтического лечения.

Ключевые слова: ортодонтическое лечение, толщина костной ткани, КЛКТ, центр сопротивления, торк зуба

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While developing an orthodontic treatment plan, a dentist should consider a number of factors that may affect the outcome, such as the age of the patient, their growth potential, sex, and health conditions. Other important factors to look at include the mechanics of tooth movement and the center of resistance, tooth inclination, tooth vitality, and the thickness of the surrounding bone tissue [1, 2]. The concept of the center of resistance was first introduced in 1917 by Fish, who proposed that there is a point in a free object through which an applied force will pass to move the object linearly without rotating it; this point is a point of equilibrium. A tooth is not a free object since it is surrounded by periodontal tissue. So, where its center of resistance is located depends on the length of its root, the number of roots it has and the amount of the surrounding bone tissue [3]. Research studies have demonstrated that the center of resistance of a single-rooted tooth surrounded by bone tissue lies 1/4–1/3 of the distance between the cementoenamel junction and the root apex [3–5].

While planning to change the vestibulo-oral inclination of a front tooth, it is important to account for the bone thickness around it [6, 7]. Accurate predictions of tooth movement will help to avoid complications that might arise in patients with insufficient bone volume. Dental radiography is a widely used imaging modality for diagnosing dental anomalies [8]. Cone-

beam computed tomography (CBCT) is a diagnostic tool that allows measuring lingual/palatal and vestibular bone thickness at different levels of the tooth root and therefore can aid orthodontic treatment planning [8]. The planned tooth movements can pose a risk for the patient in the absence of CBCT data about the thickness of bone tissue surrounding the root [9–11]. Using CBCT images of the front teeth and the technique proposed in this article, an orthodontist can accurately determine the degree of inclination and the position of incisors, measure the amount of bone surrounding the root, predict the ultimate position of the root after tooth movement and thus avert some complications associated with bone tissue deficit.

The aim of the study was to develop an original universal CBCT-based technique for measuring bone thickness around the front teeth that can be employed to safely change the vestibulo-oral inclination (torque) of the front teeth.

METHODS

The analysis focused on teeth 1.3–2.3 and 3.3–4.3 in the frontal segment. A total of 106 CBCT images of dentistry patients aged 20 to 35 years were analyzed. The following inclusion criteria were applied: dental or jaw bone abnormalities in the frontal segment in the sagittal plane; absence of cardiovascular or endocrine disorders; absence of blood disorders. Exclusion criteria: age below 20 and above 35 years, pregnancy, breastfeeding, somatic pathology, cardiovascular, endocrine or blood disorders, systemic osteoporosis, smoking. Of all the participants, 45 (44.6%) were male and 61 (55.4%) were female. The analyzed CBCT images were sorted into 3 groups: images showing normally inclined maxillary and mandibular incisors (group 1), images suggestive of maxillary/mandibular incisor protrusion, and images suggestive of maxillary/mandibular incisor retrusion. To investigate the relationship between the pathologic inclination of the teeth and the thickness of the cortical plate, measurements were taken at the level of the cervical, middle and apical thirds of the root on the vestibular and lingual/palatal sides. CBCT was performed using a Planmeca ProMax 3DMid Ceph imaging unit (Planmeca Oy; Finland) with the patient's head positioned vertically. Field-of-view centering was carried out using visual light landmarks. The field of view covered the maxilla, the mandible, the maxillary sinus, and the orbit. Scanner settings: tube voltage of 90 kV, tube current of 12.5 mA. The minimal informative slice thickness was 0.2 mm; voxel size was 200 μm, effective dose was 90 μSv. The field of view (FOV) size was 16 × 16 cm.

Obviously, the quality of CT images is superior to that of cephalograms in terms of edge sharpness. CT scans enable more accurate angular and linear measurements using landmark positions. For the purpose of the study, we selected sagittal-plane fragments on the obtained 16 ×16 images. Then, using the Schwartz method, the maxillary (NL) and mandibular (ML) planes were delineated, and the inclination and position of incisors were defined as the angle between the long axis of the tooth and the maxillary/mandibular planes. The bottom outer and the inner upper angles were studied on the maxilla and mandible, respectively. For maxillary incisors, the angle of 70° + 5° was considered normal. The angle ≤70° was interpreted as incisor protrusion; the angle ≥75° was interpreted as incisor retrusion. For mandibular incisors, the angle of 90° + 5 was interpreted as normal. The angle < 90° was interpreted as retrusion, and the angle > 95° was interpreted as protrusion. The obtained data were added to the patient's medical record.

The data were processed in Microsoft EXCEL and STATGRAPHICS Plus 5.1. We calculated the arithmetic mean (M), the error of the mean (± *m*), the mean arithmetic norm (M), and the error of the norm (± *m*). The differences were considered significant at $p < 0.05$.

RESULTS

The following measurements were performed using the CBCT images of the front teeth:

1) for maxillary incisors, vestibular bone thickness at the cervical third of the root was measured as a distance between the outer cortical plate (Av) and the outer surface of the cervical third of the root (Bv); for mandibular incisors, vestibular bone thickness was measured as a distance between the outer cortical plate (Gv) and the outer surface of the cervical third of the root (Hv) (Fig. 1);

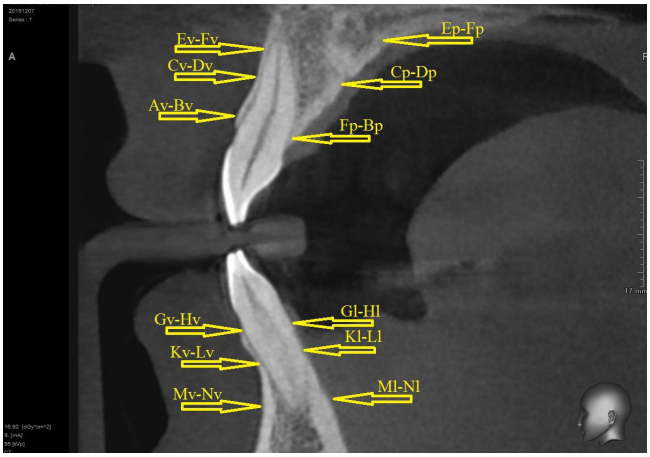

Fig. 1. Measurements on the upper and lower jaws

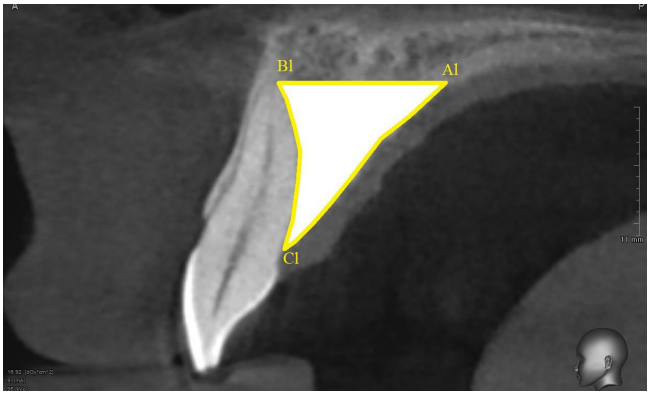

Fig. 2. Bone tissue area measurement

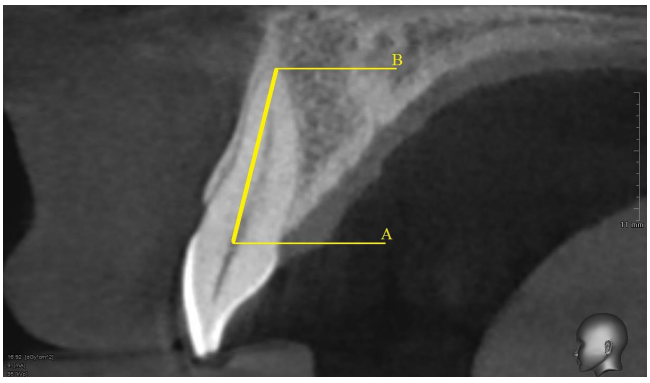

Fig. 3. Root-length measurement

2) for maxillary incisors, vestibular bone thickness at the middle third of the root was measured as a distance from the outer cortical plate (Cv) to the outer surface of the middle third of the root (Dv); for mandibular incisors, vestibular bone thickness was measured as a distance between the outer cortical plate (Kl) and the outer surface of the middle third of the root (Lv) (see Fig. 1);

3) for maxillary incisors, vestibular bone thickness at the apical third of the root was measured as a distance between the root apex (Ev) and the outer cortical plate (Fv); for mandibular incisors, the distance was measured from the root apex (Mv) to the outer cortical plate (Nv) (see Fig.1);

4) for maxillary incisors, palatal bone thickness at the cervical third of the root was measured as a distance from the outer cortical plate (Fp) to the lingual surface of the tooth root (Bp) in the cervical region; for mandibular incisors, the distance was measured between the outer cortical plate (Gl) and the lingual surface of the cervical third of the root (Hl) (see Fig. 1);

5) for maxillary incisors, palatal bone thickness at the middle third of the root was measured as a distance between the outer cortical plate (Cp) and the lingual surface of the middle third of the root (Dp); for mandibular incisors, the distance was measured from the outer cortical plate (Kl) to the lingual surface of the middle third of the root (Ll) (see Fig. 1);

6) for maxillary incisors, palatal bone thickness at the apical third of the root was measured as a distance between the tooth apex (Ep) and the outer cortical plate (Fp); for mandibular incisors, the distance was measured between the root apex (Ml) and the outer cortical plate (Nl) (see Fig. 1);

7) the area of lingual and palatal bone tissue was calculated as an area enclosed by the lines passing from the outer cortical plate at the root apex (Al) to the root apex (Bl) to the upper (palatal) arch (Cl) (Fig. 2);

8) the length of the root was measured as a distance from its anatomical cervix (A) to the root apex (B) (Fig. 3);

9) the height of the interdental septum was measured as a distance between the septal peak (A) and the perpendicular connecting the apices of the two adjacent teeth (B) (Fig. 4);

10) The degree of front teeth protrusion or retrusion was assessed from the obtained cephalometric radiography data: the angle U1–NL on the maxilla, where U1 is the line passing through the incisor long axis to the maxillary base plane (NL); and the angle L1–ML on the mandible, where L1 is the line passing through the long axis of the mandibular incisor to the mandibular base plane (ML).

Below, we offer a schematic representation of tooth movement in the upper and lower jaw bones for protruded and retruded teeth (Fig. 5, 6).

Schematic representation of maxillary incisor retrusion

The difference in angulation (a retruded vs normal tooth position) can be overcome by rotation around the fixedpoint O (the point of resistance); O needs to be linearly moved in the cervical and apical thirds of the root to a distance that offsets the difference in angulation expressed in degrees (Fig. 6). Given that BA AC and DC CA, the linear movement in the cervical third of the root can be described as follows:

$$\text{Thickness}\ {}^{\text{Maxilla}\ (1/3)}_{\text{vest}} = \frac{1}{3} L_{\text{корн}} \times \tan\alpha, \text{where}\ \alpha = \angle BOA,$$

the linear movement in the apical region can be described using the formula:

$$\text{Thickness}\ {}^{\text{Maxilla}\ (3/3)}_{\text{vest}} = \frac{2}{3} L_{\text{корн}} \times \tan\alpha, \text{where}\ \alpha = \angle BOA.$$

Retrusion of maxillary incisor movement can be described in the similar manner.

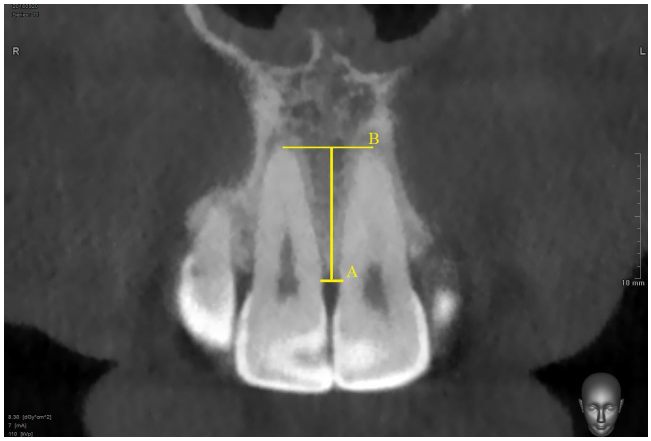

Fig. 4. Measurement of interdental septum height

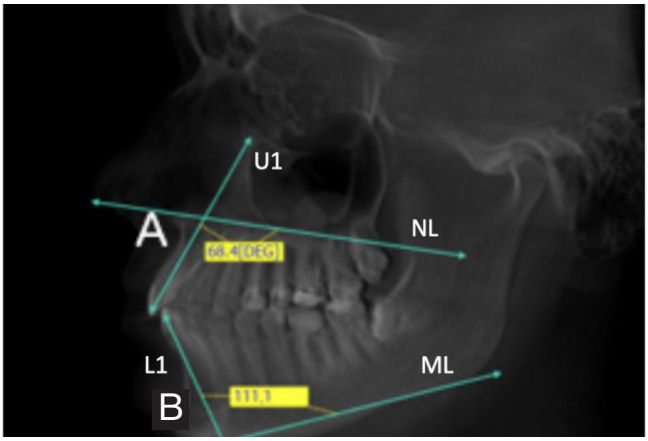

Fig. 5. Measurement of mandibular and maxillary incisor inclination

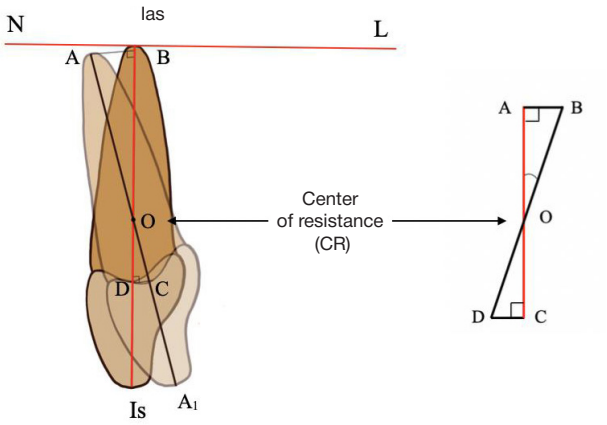

Fig. 6. The schematic representation of maxillary linear tooth movement in the surrounding bone tissue in the event of retrusion. Is is the cutting edge of the maxillary incisor; las is the apex of the maxillary incisor; las–Is is the incisor long axis; NL is a line in the maxillary plane; ∠Is–las–NL is the angle of maxillary incisor inclination; BD is a long tooth axis in the event of a normal (standard) incisor position; AA_1 is the long axis of a retruded incisor (actual position); O is the center of resistance; ∠BOA is an angle between the normal (standard) and actual axes; ∠COD is an angle between the normal (standard) and actual axes; AC means that the line BA is perpendicular to the line AC; ∠ Is–las –L (∠Is–las to NL) is an angle that characterizes the normal position of the incisor relative to the maxillary plane NL; las–Is is the long axis of a normally positioned (inclined) incisor; AA_1 is the long axis of a retruded incisor; point B is the projection of the apical point A of a retruded maxillary incisor to the axis of a normally inclined tooth; point D is the projection of the deepest point of the cementoenamel junction in the upper cervical region of the root of a retruded incisor to the long axis 12 of a normally inclined incisor; point C is a point in which the perpendicular drawn from the deepest point of the cementoenamel junction in the upper cervical region of a retruded tooth to the tooth axis of a normally inclined incisor and the line AA 1; O is the center of resistance; ∠BOA = ∠COD is an angle between the normal (standard) and actual axes; triangles AOB and COD are right-angled triangles

Table 1. The universal table of linear tooth movement in the bone (mm) depending on tooth inclination (degrees)

	1°	2°	3°	4°	5°	6°	7°
	9 ⩽ L* < 10						
1/3	[0,052…0,058]	[0,105…0,116]	[0,157…0,175]	[0,21…0,233]	[0,262…0,292]	[0,315…0,35]	[0,368…0,409]
3/3	[0,105…0,116]	[0,21…0,233]	[0,314…0,349]	[0,42…0,466]	[0,525…0,583]	[0,631…0,701]	[0,737…0,819]
	10 ⩽ L < 11						
1/3	[0,058…0,064]	[0,116…0,128]	[0,175…0,192]	[0,233…0,256]	[0,292…0,321]	[0,35…0,385]	[0,409…0,45]
3/3	[0,116…0,128]	[0,233…0,256]	[0,349…0,384]	[0,466…0,513]	[0,583…0,642]	[0,701…0,771]	[0,819…0,9]
	11 ⩽ L < 12						
1/3	[0,064…0,07]	[0,128…0,14]	[0,192…0,21]	[0,256…0,28]	[0,321…0,35]	[0,385…0,42]	[0,45…0,491]
3/3	[0,128…0,14]	[0,256…0,279]	[0,384…0,419]	[0,513…0,559]	[0,642…0,7]	[0,771…0,841]	[0,9…0,982]
	12 ⩽ L < 13						
1/3	[0,07…0,076]	[0,14…0,151]	[0,21…0,227]	[0,28…0,303]	[0,35…0,379]	[0,42…0,455]	[0,491…0,532]
3/3	[0,14…0,151]	[0,279…0,303]	[0,419…0,454]	[0,559…0,606]	[0,7…0,758]	[0,841…0,911]	[0,982…1,064]
	13 ⩽ L < 14						
1/3	[0,076…0,081]	[0,151…0,163]	[0,227…0,245]	[0,303…0,326]	[0,379…0,408]	[0,455…0,49]	[0,532…0,573]
3/3	[0,151…0,163]	[0,303…0,326]	[0,454…0,489]	[0,606…0,653]	[0,758…0,817]	[0,911…0,981]	[1,064…1,146]
	14 ⩽ L < 15						
1/3	[0,081…0,087]	[0,163…0,175]	[0,245…0,262]	[0,326…0,35]	[0,408…0,437]	[0,49…0,526]	[0,573…0,614]
3/3	[0,163…0,175]	[0,326…0,349]	[0,489…0,524]	[0,653…0,699]	[0,817…0,875]	[0,981…1,051]	[1,146…1,228]
	15 ⩽ L < 16						
1/3	[0,087…0,093]	[0,175…0,186]	[0,262…0,28]	[0,35…0,373]	[0,437…0,467]	[0,526…0,561]	[0,614…0,655]
3/3	[0,175…0,186]	[0,349…0,372]	[0,524…0,559]	[0,699…0,746]	[0,875…0,933]	[1,051…1,121]	[1,228…1,31]
	16 ⩽ L < 17						
1/3	[0,093…0,099]	[0,186…0,198]	[0,28…0,297]	[0,373…0,396]	[0,467…0,496]	[0,561…0,596]	[0,655…0,696]
3/3	[0,186…0,198]	[0,372…0,396]	[0,559…0,594]	[0,746…0,793]	[0,933…0,992]	[1,121…1,191]	[1,31…1,392]
	17 ⩽ L < 18						
1/3	[0,099…0,105]	[0,198…0,21]	[0,297…0,314]	[0,396…0,42]	[0,496…0,525]	[0,596…0,631]	[0,696…0,737]
3/3	[0,198…0,209]	[0,396…0,419]	[0,594…0,629]	[0,793…0,839]	[0,992…1,05]	[1,191…1,261]	[1,392…1,473]
	18 ⩽ L < 19						
1/3	[0,105…0,111]	[0,21…0,221]	[0,314…0,332]	[0,42…0,443]	[0,525…0,554]	[0,631…0,666]	[0,737…0,778]
3/3	[0,209…0,221]	[0,419…0,442]	[0,629…0,664]	[0,839…0,886]	[1,05…1,108]	[1,261…1,331]	[1,473…1,555]
	19 ⩽ L < 20						
1/3	[0,111…0,116]	[0,221…0,233]	[0,332…0,349]	[0,443…0,466]	[0,554…0,583]	[0,666…0,701]	[0,778…0,819]
3/3	[0,221…0,233]	[0,442…0,466]	[0,664…0,699]	[0,886…0,932]	[1,108…1,167]	[1,331…1,401]	[1,555…1,637]

Note: L is root length.

Schematic representation of mandibular incisor retrusion

The linear movement of mandibular incisors in the event or protrusion or retrusion are described using the same approach, but the mandible is designated as ML (Fig. 7).

RESULTS

Using the CBCT images of patients with normally inclined teeth, we discovered that vestibular bone thickness at the cervical third of the root of the maxillary central incisors was 1.04 ± 0.04 mm for tooth 1.1 and 0.96 ± 0.07 mm for tooth 2.1. For the maxillary lateral incisors, the figures were as follows: 0.81 ± 0.04 mm for tooth 1.2 and 0.84 ± 0.09 mm for tooth 2.2. For the maxillary canines, the following values were obtained: 0.91 ± 0.06 mm for tooth 1.3 and 0.84 ± 0.09 mm for tooth

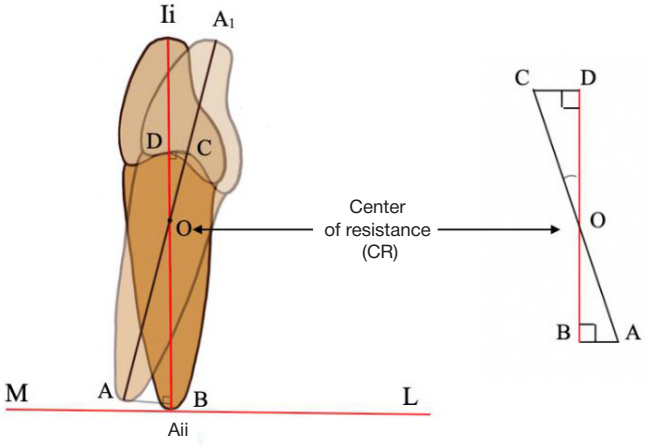

Fig. 7. Schematic representation of mandibular incisor retrusion

Table 2. The universal table of linear tooth movement in the bone (mm) depending on tooth inclination (degrees)

	8°	9°	10°	11°	12°	13°	14°	15°
	9 ⩽ L* < 10							
1/3 vest	[0,422…0,468]	[0,475…0,528]	[0,529…0,588]	[0,583…0,648]	[0,638…0,709]	[0,693…0,77]	[0,748…0,831]	[0,804…0,893]
3/3 vest	[0,843…0,937]	[0,95…1,056]	[1,058…1,176]	[1,166…1,296]	[1,275…1,417]	[1,385…1,539]	[1,496…1,662]	[1,608…1,786]
	10 ⩽ L < 11							
1/3 vest	[0,468…0,515]	[0,528…0,581]	[0,588…0,647]	[0,648…0,713]	[0,709…0,779]	[0,77…0,847]	[0,831…0,914]	[0,893…0,982]
3/3 vest	[0,937…1,031]	[1,056…1,161]	[1,176…1,293]	[1,296…1,425]	[1,417…1,559]	[1,539…1,693]	[1,662…1,828]	[1,786…1,965]
	11 ⩽ L < 12							
1/3 vest	[0,515…0,562]	[0,581…0,634]	[0,647…0,705]	[0,713…0,778]	[0,779…0,85]	[0,847…0,923]	[0,914…0,997]	[0,982…1,072]
3/3 vest	[1,031…1,124]	[1,161…1,267]	[1,293…1,411]	[1,425…1,555]	[1,559…1,7]	[1,693…1,847]	[1,828…1,995]	[1,965…2,144]
	12 ⩽ L < 13							
1/3 vest	[0,562…0,609]	[0,634…0,686]	[0,705…0,764]	[0,778…0,842]	[0,85…0,921]	[0,923…1]	[0,997…1,08]	[1,072…1,161]
3/3 vest	[1,124…1,218]	[1,267…1,373]	[1,411…1,528]	[1,555…1,685]	[1,7…1,842]	[1,847…2,001]	[1,995…2,161]	[2,144…2,322]
	13 ⩽ L < 14							
1/3 vest	[0,699...0,795]	[0,686…0,739]	[0,764…0,823]	[0,842…0,907]	[0,921…0,992]	[1…1,077]	[1,08…1,164]	[1,161…1,25]
3/3 vest	[1,218…1,312]	[1,373…1,478]	[1,528…1,646]	[1,685…1,814]	[1,842…1,984]	[2,001…2,155]	[2,161…2,327]	[2,322…2,501]
	14 ⩽ L < 15							
1/3 vest	[0,656…0,703]	[0,739…0,792]	[0,823…0,882]	[0,907…0,972]	[0,992…1,063]	[1,077…1,154]	[1,164…1,247]	[1,25…1,34]
3/3 vest	[1,312…1,405]	[1,478…1,584]	[1,646…1,763]	[1,814…1,944]	[1,984…2,126]	[2,155…2,309]	[2,327…2,493]	[2,501…2,679]
	15 ⩽ L < 16							
1/3 vest	[0,703…0,75]	[0,792…0,845]	[0,882…0,94]	[0,972…1,037]	[1,063…1,134]	[1,154…1,231]	[1,247…1,33]	[1,34…1,429]
3/3 vest	[1,405…1,499]	[1,584…1,689]	[1,763…1,881]	[1,944…2,073]	[2,126…2,267]	[2,309…2,463]	[2,493…2,659]	[2,679…2,858]
	16 ⩽ L < 17							
1/3 vest	[0,75…0,796]	[0,845…0,898]	[0,94…0,999]	[1,037…1,101]	[1,134…1,204]	[1,231…1,308]	[1,33…1,413]	[1,429…1,518]
3/3 vest	[1,499…1,593]	[1,689…1,795]	[1,881…1,998]	[2,073…2,203]	[2,267…2,409]	[2,463…2,617]	[2,659…2,826]	[2,858…3,037]
	17 ⩽ L < 18							
1/3 vest	[0,796…0,843]	[0,898…0,95]	[0,999…1,058]	[1,101…1,166]	[1,204…1,275]	[1,308…1,385]	[1,413…1,496]	[1,518…1,608]
3/3 vest	[1,593…1,686]	[1,795…1,901]	[1,998…2,116]	[2,203…2,333]	[2,409…2,551]	[2,617…2,77]	[2,826…2,992]	[3,037…3,215]
	18 ⩽ L < 19							
1/3 vest	[0,843…0,89]	[0,95…1,003]	[1,058…1,117]	[1,166…1,231]	[1,275…1,346]	[1,385…1,462]	[1,496…1,579]	[1,608…1,697]
3/3 vest	[1,686…1,78]	[1,901…2,006]	[2,116…2,233]	[2,333…2,462]	[2,551…2,692]	[2,77…2,924]	[2,992…3,158]	[3,215…3,394]
	19 ⩽ L < 20							
1/3 vest	[0,89…0,937]	[1,003…1,056]	[1,117…1,176]	[1,231…1,296]	[1,346…1,417]	[1,462…1,539]	[1,579…1,662]	[1,697…1,786]
3/3 vest	[1,78…1,874]	[2,006…2,112]	[2,233…2,351]	[2,462…2,592]	[2,692…2,834]	[2,924…3,078]	[3,158…3,324]	[3,394…3,573]

Table 3. Comparison of alveolar bone parameters surrounding the tooth 3.3. in the events of its protrusion and retrusion

2.3. Vestibular bone thickness at the apical third of the root of the maxillary central incisors was as follows: 0.95 ± 0.04 mm for tooth 1.1, 0.71 ± 0.04 mm for tooth 2.1, 1.05 ± 0.06 mm for tooth 1.2, 1.31 ± 0.08 mm for tooth 2.2, 1.22 ± 0.06 mm for tooth 1.3, 1.31 ± 0.08 mm for tooth 2.3. Vestibular bone thickness at the cervical third of mandibular incisors was 1.12 ± 0.04 mm for tooth 3.1, 1.26 ± 0.06 mm for tooth 4.1, 0.89 ± 0.07 mm for tooth 3.2, 1.18 ± 0.03 mm for tooth 4.2, 0.94 ± 0.03 mm for tooth 3.3, 1.26 ± 0.12 mm for tooth 4.3. At the apical third, bone thickness was 3.35 ± 0.04 mm for tooth 3.1, 2.44 ± 0.04 mm for tooth 4.1, 2.86 ± 0.05 mm for tooth 3.2, 2.88 ± 0.07 mm for tooth 4.2, 3.53 ± 0,21 mm for tooth 3.3, 2.81 ± 0.06 mm for tooth 4.3.

Thus, the CBCT images of pathologically inclined teeth revealed that the most pronounced bone deficit was observed in the maxilla around the cervical third of the root of the protruded central and lateral incisors (20% and 16%, respectively, relative to the control group). In the mandible, bone deficit around the apical third of the protruded central and lateral incisors was 64% and 16%, respectively. For the canine teeth, bone deficit was 22% relative to the control group. Bone thickness around the cervical third of the root of the retruded maxillary frontal teeth was 36% less than in the control group; for the maxillary lateral incisors, bone deficit reached 24%. For the maxillary canine teeth, alveolar bone thickness around the cervical third of the root was 31% less than in the control group. In the mandible, vestibular bone deficit around the cervical third of the root was 27%, 38.5% and 33% for the central incisors, lateral incisors and canine teeth, respectively.

Thus, both vestibular and palatal bone deficit was observed at the cervical third of the root for two groups of upper teeth, being the most pronounced around the retruded incisors. In the mandible, the loss of vestibular bone thickness at the cervical third of the retruded frontal teeth was more pronounced; however, the loss of lingual bone thickness at the cervical third of the root was more pronounced for protruded vs. retruded

Area of measurements (mm)	Protrusion	Retrusion	Significance
BT vest. 1/3	0,73 ± 0,10	0,31 ± 0,05	$p < 0,001$
BT vest. 3/3	3,39 ± 0,20	2,97 ± 0,12	$p > 0,05$
L_{root}	13,88 ± 0,24	16,71 ± 0,19	$p < 0,001$

Note: BT stands for bone thickness; $p < 0.05$ indicates statistically significant differences.

teeth. The detected significant differences in vestibular bone thickness at the cervical third of the root of the studied frontal teeth suggest that there are potential risk areas of bone loss in patients with retrusion and protrusion undergoing orthodontic treatment.

Tables 1 and 2 summarize the results of our study and contain information about the root length for the maxillary and mandibular frontal teeth, as well as vestibular and palatal/lingual bone thickness at the cervical and apical thirds of their roots. The table allows estimating the bone volume needed to change tooth inclination from 1° to 15°. Our calculations rely on the root length of a studied tooth as a starting point. While planning a desired change in tooth inclination, an orthodontist can use the data from the Tables to make sure that there is sufficient bone volume around the cervical and apical thirds of the root.

Information provided in the Tables will help to avoid bone resorption in the area of bone deficit during orthodontic treatment and prevent the root from getting beyond the cortical bone.

How to use the Tables:

1) measure the length of the tooth root and the vestibular/palatal bone thickness at the cervical and apical thirds;

2) consult the table for the recommended bone thickness calculated for the already changed vestibulo-oral tooth inclination;

3) using the obtained data, plan the movement of the frontal teeth.

Below, we provide an example of changes to the vestibulo-oral inclination of the tooth 3.3. Information about bone thickness for a protruded or retruded tooth 3.3 is provided in the Tables. Table 3 shows the results of measurements for this tooth: root length (L, mm), vestibular bone thickness at the cervical third of the root (TKT vest. 1/3, mm), and vestibular bone thickness at the apical third (TKT vest. 3/3, mm).

Thus, when using analyzing bone thickness around the cervical third of the root of tooth 3.3. in the event of protrusion or retrusion, one can plan a safe change to tooth inclination by consulting the Tables, which demonstrate that the length of the root falls withing the range from 13 to 14 mm. So, the inclination of the tooth 3.3. can be safely changed by 9o, considering the amount of bone around the cervical third of the root. For this tooth, there is no bone deficit around the apical third of the root. For the retruded tooth 3.3, the length of the root will range from 16 to 17 mm, so one can safely change the inclination of this tooth by 3o; creating a more pronounced inclination is prevented by bone deficit in this area. Bone thickness is sufficient around the apical third of the root.

DISCUSSION

There is a wealth of literature describing the thickness of the cortical and alveolar bones in patients with pathologically inclined teeth. A 2007 study used CT images to describe the position of mandibular incisors, their root apices and the condition of bone tissue in patients with retrusive occlusion and pathological tooth inclination. The researchers measured distances from the root apex to the inner contour of the cortical bone on the vestibular and lingual surfaces, tooth inclinations and the thickness of the surrounding bone. They concluded that there was an association between the vestibular bone angle and tooth inclination, the lingual bone angle and the incisor inclination angle [12]. In 2009, another team of researchers studied dental bone thickness; however, they did not report the association between the incisor angle and the distance from the vestibular and lingual cortical plates to root apices. The study confirms the association between incisor inclination and the position of tooth apices, as well as the morphology of the bone surrounding the tooth [13].

CONCLUSIONS

CBCT has a better diagnostic capacity, with its minimal slice thickness of 0.2 mm, than multislice spiral CT, which offers the slice thickness of only 1 mm and therefore is diagnostically unacceptable when it comes to dentofacial examination. The effective dose delivered by CBCT is 61–134 μSv; the effective dose during an orthopantomography scan is 4 times lower, whereas the effective dose delivered by multislice spiral CT is 1.5–12.3 times higher than during a CBCT scan. CBCT is an advantageous diagnostic modality in terms of contour and object sharpness and enables more accurate linear and angular measurements using landmark positions. Our universal tables allow the orthodontist to estimate bone thickness at different levels of the tooth root in order to ensure a safe change in tooth inclination in patients with pathologically inclined teeth. When used in combination, our tables and CBCT make it possible to assess the safety of tooth movement and the necessary thickness of bone tissue at different levels of the tooth root. The tables are a huge aid in dental diagnosis and orthodontic treatment planning, helping to assess the safety of movement frontal teeth depending on the thickness of the surrounding bone.

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ANALYSIS OF OSSEOUS TISSUE TEMPERATURE DURING PREPARATION OF RECIPIENT BED FOR CYLINDRICAL IMPLANTS

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In the early 21st century, robot-assisted dental implant surgery became a popular alternative to classic implant placement protocols. Postoperative complications are often provoked by poor compliance with surgical guidelines for implant placement, resulting in the overheating of the osteotomy site. The aim of this study was to measure the temperature of osseous tissue at the dental implant site during classic and robot-assisted dental implant placement performed at different cooling modes. Avital skeletonized mandibles of Vietnamese pot-bellied pigs were used as an experimental model. The recipient bed was prepared following the classic surgical protocol. Three cooling modes were tested: no irrigation, irrigation with sterile saline at 25–30 ml/min and standard 75 ml/min irrigation recommended by the standard surgical protocol. The temperature of the isotonic solution was 25 °C. The study showed that both classic and robot-assisted dental implant placement techniques are safe if there is sufficient irrigation and good compliance with the surgical protocol.

Keywords: robot-assisted system, bone heating, dental implant placement

Author contribution: Ivashchenko AV, Postnikov MA collected and analyzed clinical data; Tlustenko VP did organizational work and conducted the experiment; performed systematic analysis; Popov NV performed statistical analysis of the obtained data; Yablokov AE did organizational work and conducted the experiment, collected and analyzed clinical data; Tlustenko VS analyzed clinical data. Tugushev VV, Cherezova NI, Mukhina AA did organizational work and conducted the experiment; Belanov GN — sistematic analysis.

Compliance with ethical standards: the study was approved by the Ethics Committee of Samara State Medical University (Protocol 209 dated February 3, 2021).

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ОЦЕНКА ТЕРМОМЕТРИЧЕСКИХ ДАННЫХ КОСТНОЙ ТКАНИ ПРИ ФОРМИРОВАНИИ ВОСПРИНИМАЮЩЕГО МАТЕРИНСКОГО ЛОЖА ПОД ЦИЛИНДРИЧЕСКИЕ ДЕНТАЛЬНЫЕ ИМПЛАНТАТЫ

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Установка дентальных имплантатов с применением механотронных устройств получила широкое применение в начале XXI в., наряду с классическим имплантологическим протоколом. Осложнения нередко обусловлены несоблюдением стандарта хирургического протокола проведения дентальной имплантации и, как следствие, повышением фокуса температуры в месте сверления костной ткани челюстей. Целью работы было оценить фокус температуры костной ткани в области установки дентального имплантата, проводимой по классической методике и с применением механотронной системы с различной степенью охлаждения. В качестве экспериментальной модели использовали авитальную скелетированную нижнюю челюсть поросенка вьетнамской вислобрюхой породы. В соответствии с классическим хирургическим протоколом было сформировано воспринимающее материнское ложе. Исследование проводили в трех режимах: при отсутствии подачи изотонического раствора, при незначительном его объеме (25–30 мл/мин) и при орошении раствором в соответствии с хирургическим протоколом (75 мл/мин). Температура подаваемого изотонического раствора составляла 25 °С. По результатам исследования, и классическая методика установки цилиндрических дентальных имплантатов, и их инсталляция с применением механотронной системы безопасны при условии соблюдения хирургического протокола и с достаточным объемом подаваемого изотонического раствора.

Ключевые слова: механотронная система, нагревание кости, дентальная имплантация

Вклад авторов: А. В. Иващенко — сбор и анализ клинических данных; В. П. Тлустенко — подготовка клинического материала, систематический анализ; М. А. Постников — сбор и анализ клинических данных; Н. В. Попов — обработка и анализ полученного материала; А. Е. Яблоков — подготовка клинического материала, сбор и анализ клинических данных; В. С. Тлустенко — анализ клинических данных; В. В. Тугушев — подготовка клинического материала; Н. И. Черезова — подготовка клинического материала; А. А. Мухина — подготовка клинического материала. Г. Н. Беланов — систематический анализ.

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Robot-assisted dental implant surgery has become a popular alternative to classic implant placement protocols [1, 2]. The key risk factor for postoperative complications is poor compliance with surgical guidelines for implant placement, resulting in the overheating of the osteotomy site [3, 8]. Heating to over 48 °C causes a thermal burn to stromal dental tissue and protein denaturation. This prevents osseointegration between the implant and the bone and eventually leads to implant failure [4, 11].

The primary cause of an osseous burn during drilling is the wrong choice of the bone cutting and cooling modes (drilling speed >1,200 rpm, saline irrigation rate < 70 ml/min) [5, 9, 10]. During robot-assisted implant placement, saline irrigation must be performed at 100 ml/min. Drill manufacturers (Dentium, Israel; MIS, Israel; etc.) recommend that the maximum number of drill uses during surgery should be limited to 40–45 [12–15]. Discrete irrigation of the surgical field during recipient bed preparation is not allowed in both classic or robot-assisted dental implant surgery [6–8].

The aim of this study was to measure the temperature of osseous tissue at the dental implant site during classic and robot-assisted dental implant placement performed at different cooling modes.

METHODS

The study investigated the response of the alveolar bone to osteotomy and was carried out in 15 Vietnamese pot-bellied pigs. Dental implants used in the study were cylindrical. The recipient bed was prepared by drilling using an optic implant handpiece fixed in the robotic arm; the drilling speed was 800 to 1,500 rpm.

In all 3 experimental groups, irrigation of the osteotomy site was performed according to the guidelines for classic dental implant surgery. Implants were inserted at 30–45 Ncm torque. A total of 30 3.6 × 8.0 mm cylindrical dental implants (Dentium®; Israel) were installed.

The animals were divided into 3 groups based on the drilling speed and the type of surgical instrument (Table 1).

The choice of implant placement sites was based on the similarity between jaw bone density in humans and the animals used in our experiment.

Before proceeding to the main part of the study, we computed the maximum optimal temperature during osteotomy for implant placement in ANSYS 19.2 (Cadfem Company; USA) (see Fig.).

The temperature of the bone matrix during osteotomy was modeled at 3 sites: the site of bur tip/bone contact, in the apical alveolar area of the prepared recipient bed and in the recipient bed immediately after removing the bur.

While modeling the temperature during osteotomy, the pressure of the drill on the bone and drilling time were assumed to be constant.

Our assessments of thermodynamic osseous tissue states confirm that an increase in the drilling speed to 1,500 rpm provokes a proportional rise in t °C to the critical threshold of 60.2 °C, given that other drilling parameters remain unchanged. An increase in the drilling speed to 1,500 rpm results in the maximum temperature gradient rising from 37.6 °C to 60.2 °C (i.e., by 22.6 °C).

Three cooling modes were tested: no irrigation, irrigation with sterile saline at 25–30 ml/min and standard 75 ml/min irrigation recommended by the standard surgical protocol. The temperature of the isotonic solution was 25 °C.

We also studied the response of porcine mandibular bone tissue to robot-assisted osteotomy using the device proposed in [16]. For the "robotic" part of the experiment, similar cylindrical dental implants (Dentium; Korea) were installed using a Surgic XT Plus unit with an optic implant handpiece (Japan). The following cooling modes were tested: 75 ml/min irrigation with sterile saline recommended by the standard surgical protocol, 30 ml/min irrigation and no irrigation. The temperature of the isotonic solution was 25 °C.

The pilot drill was installed in the optic handpiece; the latter was fixed in the robotic arm. Using the joystick control, the surgeon positioned the drill at the drilling site the preoperative road map. Then, the protocol for automated bone drilling was activated. Once the drilling was finished, the robotic arm retrieved the pilot drill from the mouth cavity and the pilot drill was replaced with the bed formation drill.

Temperature was measured using a Testo 104-ir infrared probe thermometer (Testo AG; Germany). Prior to the experiment, the skeletonized porcine mandible was exposed to distilled water (t = 45 ° C) for 10 min. The temperature of the osteotomy site before drilling was 36.8 °C.

The majority of the installed implants were in quadrants 3 (6 implants, 60%) and 4 (4 implants, 40%) in group 1; quadrants 3 (7 implants, 70%) and 4 (3 implants, 30%) in group 2; quadrants 3 (5 implants, 50%) and 4 (5 implants, 50%) in group 3.

During the experiment on the mandibular bone tissue of pigs, we took temperature measurements of the bone matrix at the osteotomy site. For the cooling mode 3, the peak temperature was as high as 61.5 °C. For the cooling mode 2, the maximum temperature was 52 °C. The optimal temperature (39.1 °C) was achieved in the cooling mode 1.

Prior to the experiment, the animals' body temperature was taken, showing an average of 38.5 to 39.1 °C.

Table 2 shows the drilling speed, the peak temperature during drilling and the duration of drilling at different cooling modes.

The average temperature at the osteotomy site during recipient bed preparation was 39.1 °C. For our experimental animals, this value was close to normal temperature. Bur temperatures over 60 °C during osteotomy resulted in the peak bone temperature well above the physiological norm.

Table 1. Bone cutting modes during recipient bed preparation

Group	Instrument	Drilling speed, rpm	Number of installed dental implants	Implant sites and number (in brackets)
1	Lindemann guide drill	800	10	3.5 – 2 4.6 – 3 3.6 – 2 3.2 – 1 4.1 – 1 3.3 – 1
	Lindemann pilot drill	800		
	Harvest drill	800		
	Cortical drill	600		
	Transfer piece	30–45 Ncm		
2	Lindemann guide drill	1,200	10	3.4 – 2 4.5 – 1 3.3 – 3 3.2 – 1 4.2 – 2 3.1 –
	Lindemann pilot drill	1,200		
	Harvest drill	1,200		
	Cortical drill	1,000		
	Transfer piece	30–45 Ncm		
3	Lindemann guide drill	1,500	10	3.6 – 2 4.2 – 1 3.3 – 2 4.2 – 1 4.5 – 3 4.6 – 1
	Lindemann	1,500		
	pilot drill	1,500		
	Harvest drill	1,500		
	Cortical drill	1,200		
	Transfer piece	30–45 Ncm		

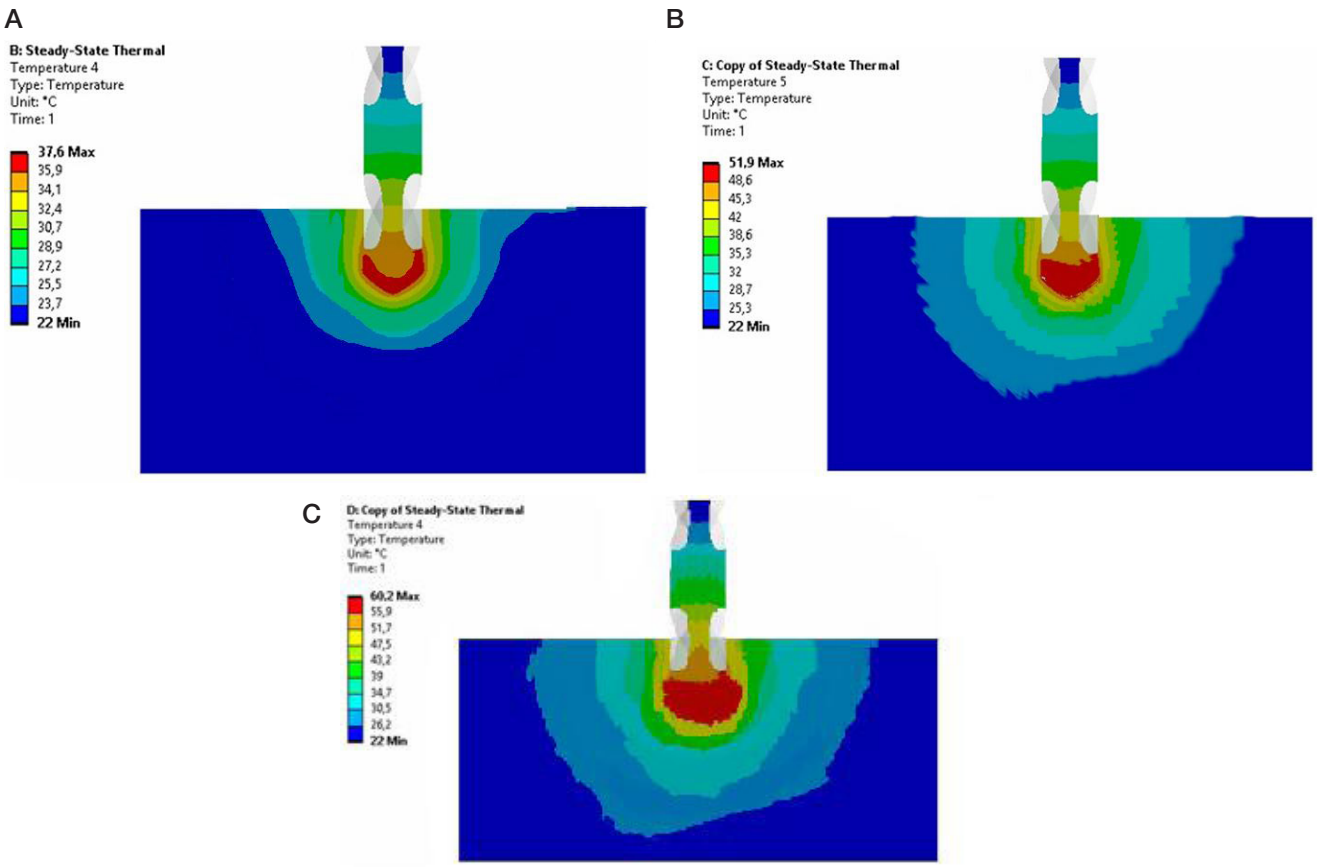

Fig. Modeled temperature during osteotomy for dental implant placement. **A**. Temperature gradient from 22 °C to 37.6 °C (800 rpm). **B**. Temperature gradient from 22 °C to 51.9 °C (1,200 rpm). **C**. Temperature gradient from 22 °C to 60,2 °C (1,500 rpm)

The relative reduction in the rate of negative outcomes in the main vs control groups, i.e relative risk reduction, was 400%. This value, along with the 95% CI, means that the effect is clinically significant (Table 3).

RESULTS

The temperature of the osteotomy site in the mandible rose to 41.3 °C from the initial value of 36.8 °C after 5 seconds of drilling in

Table 2. Peak temperature during osteotomy for dental implant surgery at different cooling modes

Cooling modes	Drilling parameters		
	Drilling speed (rpm)	Bone temperature at the osteotomy site (°C)	Drilling time (s)
Mode 1	800	39.1 ± 0.22	45
Mode 2	1,200	52 ± 0.37	45
Mode 3	1,500	61.5 ± 0.43	45

Table 3. Efficacy of dental implant surgery according to evidence-based medicine criteria

Group	Positive clinical outcome		
	Yes	No	Total
Main	5	0	5
Control	15	0	15
RO	100.00%		
RNO	20.00%		
RR	500.00%		
RRR	400.00%		
CAP	80.00%		
NNT	1.3		
Odds (main group)	–		
Odds (control group)	0.3		
OR	–		

Note: RO — rate of outcomes; RNO — rate of negative outcomes; RR — relative risk; RRR — relative risk reduction; NNT — number of patients that must be treated to prevent 1 negative outcome; OR — odds ratio.

the absence of irrigation during the classic dental implant placement procedure. Irrigation at 30 ml/min during 5 s of drilling resulted in a lower temperature at the recipient bed site (39.4 °C). With irrigation at 75 ml/min, the bone tissue temperature was 36.9 °C.

During recipient bed preparation, which involved bone drilling for 10 s, the bone matrix was heated to 51.5 °C in the absence of irrigation, 43.2 °C at < 30 ml/min irrigation and 39.6 °C at 75 ml/min irrigation recommended by the standard surgical protocol. Heating to over 45 °C causes irreversible changes to bone matrix.

Similar to the classic surgical protocol applied in the first part of the study, the robot-assisted protocol involved placement of cylindrical dental implants in the skeletonized portion of the mandible. The initial temperature of the mandible was close to human body temperature (36.8 °C). In the absence of irrigation, the bone temperature at the osteotomy site rose to 42.4 °C. When irrigation of the osteotomy site was delivered at < 30 ml/min during 5 seconds of bone drilling, the temperature of the osseous tissue at the osteotomy site was 38.2°C. With irrigation at 75 ml/min, the temperature at the osteotomy site was 37.1 °C.

During the preparation of the recipient bed, which involved drilling for 10 s, the bone matrix was heated to 53.9 °C (in the absence of irrigation), 45.7 °C (insufficient irrigation) and 38.9 °C (sufficient irrigation according to the standard surgical protocol).

DISCUSSION

At present, there is no consensus on the optimal drilling speed. According to early reports, bone temperature rose proportional to the drilling speed [17]. However, later studies revealed that this was true only for the drilling speed of 10,000 rpm [18]. None of the studies revealed any significant changes in the temperature of a human cadaver bone during drilling at 345–2,900 rpm. This means that a temperature rise was dependent on drilling pressure more than on drilling speed. It was demonstrated that low-speed, minimal-pressure drilling of bovine cortical bones caused the same rise in temperature as drilling at higher speed and higher pressure. It is high drilling speed and pressure that allow osteotomy to be performed effectively [19]. At drilling speed below 250 rpm, bone tissue gets fragmented at the edge of the defect.

CONCLUSIONS

The analysis of osseous tissue thermometry data obtained during osteotomy for the subsequent placement of cylindrical dental implants revealed that both classic and robot-assisted dental implant placement techniques can be recommended for clinical use and are sage if there is sufficient irrigation and good compliance with the surgical protocol.

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DEVELOPMENT OF MEDICAL NOMENCLATURE AND ALGORITHMS FOR DIAGNOSIS AND TREATMENT OF GOUT IN OUTPATIENT SETTINGS

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Gout is a chronic systemic disease characterized by the deposition of monosodium urate crystals in various tissues and inflammation. In Russia, time to diagnosis may be as long as 8 years. This leads to serious complications, such as urate nephropathy, and disability. Effective strategies are needed to improve the quality of medical care for gout patients. One of such strategies is creation of an expert system to aid the clinician in establishing the diagnosis and selecting adequate therapy. The cornerstone of an expert system is a knowledge base. The aim of this paper was to develop a medical nomenclature and algorithms for the diagnosis and treatment of gout that will be used to create an expert system in the future. A total of 1,174 entities were selected that laid the basis for 40 diagnostic and 50 treatment algorithms for gout patients. All informational models were verified by the expert panel.

Keywords: ontology, knowledge base, expert system, CDSS, clinical decision support system, gout

Author contribution: Osmolovsky IS built and performed technical verification of the medical nomenclature and algorithms for the diagnosis and treatment of gout, analyzed information provided by the expert panel, wrote the technical section of the paper and prepared figures and tables; Zarubina TV proposed the design of the study, supervised the study, analyzed information provided by the expert panel and performed technical verification of the informational objects; Shostak NA, Kondrashov AA, Klimenko AA collected data for the informational objects, performed clinical verification of the informational objects and wrote the clinical section of this paper.

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РАЗРАБОТКА НОМЕНКЛАТУРЫ ПОНЯТИЙ И ЛОГИЧЕСКИХ СХЕМ ПО АМБУЛАТОРНОМУ ВЕДЕНИЮ ПАЦИЕНТОВ С ПОДАГРОЙ

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Подагра является системным тофусным заболеванием, для которого характерны отложение в различных тканях кристаллов моноурата натрия и развивающееся в связи с этим воспаление. Диагностика подагры в среднем по России длится до 8 лет. Этого времени достаточно для развития различных осложнений (например, подагрической нефропатии) и инвалидизации пациента. Необходимы стратегии улучшения качества оказываемой помощи пациентам. К одной из таких стратегий можно отнести создание экспертной системы, которая могла бы помочь специалистам заподозрить подагру и рекомендовать тактику ее лечения. Основой экспертной системы является база знаний. Целью исследования было сформировать номенклатуру медицинских понятий и логических схем ведения пациентов при диагностике и лечении подагры для разработки экспертной системы. В ходе разработки номенклатуры было собрано 1174 понятия, которые легли в основу 40 логических схем по диагностике подагры и 50 логических схем лечения заболевания. Все указанные информационные модели верифицированы экспертами.

Ключевые слова: онтология, база знаний, экспертная система, система поддержки принятия врачебных решений, подагра

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Gout is a chronic systemic disease characterized by the deposition of monosodium urate crystals (tophi) in various tissues and the ensuing inflammation in patients with hyperuricemia associated with environmental and/or genetic factors. Gout affects 1–2% of the world's adult population [1]. According to clinical guidelines, gout can be effectively managed using a treat-to-target approach that consists in achieving and maintaining a target serum urate level. Sustained reduction in serum urate below 360 μmol/L results in the dissolution of monosodium urate crystals and prevents gout flares. However, delays in the initiation of urate-lowering therapy and poor adherence to treatment are common worldwide; therefore, target serum urate levels are rarely achieved, as demonstrated by laboratory tests [1]. Effective strategies are needed to improve the quality of medical care for gout patients.

In Russia, the estimated time from the first gout attack to formal diagnosis is 4–8 years [2–5]; diagnostic delays result in severe complications and comorbidities.

The timely diagnosis of gout is constrained by a number of factors, one of them being the shortage of rheumatologists at primary healthcare and other medical facilities. According to Russian health law [6], there should be at least one rheumatologist per 30,000 population. In practice, there are 0.07 rheumatologists per 10,000 population or 0.21 rheumatologists per 30,000 population, i.e. their number is

5 times lower than recommended [7, 8], hence the delays in delivering adequate medical care to gout patients.

Another problem is the lack of access to polarized light microscopy, the gold standard diagnostic test for gout [1, 9]. It is not a routine method; it cannot be used in every clinical setting and must be performed by specially trained staff [4, 9]. So, practicing physicians have little choice but to exclusively rely on clinical symptoms, which they may misinterpret during the first visit [10].

Modern technology can reduce the rate of medical errors and improve the quality of healthcare delivered to the patient. Among technological successes are diagnostic decision support systems that have been in development since the late 1960s — early 1970s [11–13]. In the past 50 years, a large pool of ready-for-use decisions has been created, many of which are still used in clinical practice. Decision support systems can be categorized in 2 groups [14]:

– systems based on machine learning: genetic algorithms, artificial neural networks, support vectors, etc. [15];

– knowledge-based systems, such as expert systems [13].

Systems that rely on machine learning (medical image processing) exploit massive databases (up to a few thousand cases) and are not required to explain the decision to the clinician have proved to be effective in many medical fields. But in the case of gout, knowledge-based systems that can operate in the absence of sufficient amount of data are more suitable. These systems are based on texts and expert knowledge and can aid the clinician in taking informed decisions [12, 13, 16].

A knowledge base is the product of knowledge engineering that develops methods for knowledge extraction, structuring and formalization. There are a few established models of knowledge representation, including production models, frames, sematic networks, etc. [12, 13, 17]. However, the modularity, ambiguity, incompleteness, and some other characteristics of a given subject area are difficult to represent using existing models [12]. To circumvent these challenges, an ontological approach can be applied; it allows developing a hierarchical nomenclature of strictly defined medical entities [12].

An ontology is a formal specification of a shared conceptual model, i.e. an abstract model of a given subject field that explicitly describes the conceptual framework of this field, is accepted by a given community and has a formal representation [12, 13]. Conventionally, ontologies are represented by semantic networks, i.e. the entirety of linked concepts; however, there are other methods of knowledge representation, including frames and production rules.

Thus, building an ontology is one of the key stages in the development of an expert system that can provide a clinician with comprehensive information about a disease. But building a medical ontology is impossible without a nomenclature of medical entities and diagnostic/treatment algorithms [12, 13]; so far, no such information objects have been constructed.

The aim of this study was to develop formal algorithms for the diagnosis and treatment of gout in an outpatient setting that could be further used to build an expert system.

METHODS

Federal clinical guidelines approved by the Russian Ministry of Health [1] were used as the main source of data for creating a medical nomenclature and diagnostic/treatment algorithms for gout. The State Registry of Medicinal Products [18] and academic publications were used as additional sources of information.

Eligibility criteria for experts participating in the development of an expert system or a similar product are not explicitly specified in Russian normative documents, so the selection was based on the formal indicators of expertise, including academic credentials, position held, and over 8 years of experience in rheumatology. The panel of experts consisted of 3 highly skilled rheumatologists from Nesterov Department of Faculty Therapy at Pirogov Russian National Research Medical University.

Diagnostic and treatment algorithms for gout were developed using MS Excel (Microsoft; USA), MS Excel Online (Microsoft; USA) and Visio (Microsoft; USA) software.

The process of developing the nomenclature of medical entities pertaining to the diagnosis and treatment of gout included the following steps:

– entities were identified using text-based methods of knowledge extraction;

– working independently — each member of the panel made corrections to the nomenclature by modifying, adding or removing the identified entities;

– the obtained results were validated by comparison and voting.

For the comparison procedure, each expert was given a nomenclature version drawn from textual sources. The members of the expert panel revised and modified the received nomenclature independent of each other. The resultant versions were compared, and the variations were brought forward for voting so as to enrich the final version of the nomenclature with new entities.

Diagnostic algorithms were developed using 2015 ACR/EULAR gout classification criteria. Treatment algorithms were based on the clinical guidelines proposed by the Russian Association of Rheumatologists [1, 19]. Diagnostic and treatment algorithms for gout were developed following the same steps as in the development of the medical nomenclature.

RESULTS

Medical nomenclature

Creating a nomenclature of medical entities pertaining to the diagnosis and treatment of gout was a multistep process that allowed us to identify 1,174 entities. As part of this process, we were faced with the need to formulate a number of requirements in order to keep the nomenclature from overgrowing and to get rid of some nomenclature items that were not used in decision making. Briefly, we had to

1. Identify entities related to the diagnosis and treatment of gout only. Entities not related to gout were not included in the nomenclature. For example, the entities “heartburn” or “gastric ulcer” are not used to diagnose or treat gout, so they were not included in the nomenclature.

2. Extract revised terminology. For example, the term “urate”/“uric acid” was not included in the nomenclature because it lacks accuracy: uric acid can be measured in various bodily fluids like urine, synovial fluid etc., and its reference levels used for diagnostic or monitoring purposes are different. Therefore, a more precise term should be used, i.e. “serum urate”. By mapping the studied terms to the SNOMED databased, we were able to refine a number of terms.

3. Arrange synonyms into groups. The terminology that describes a specific phenomenon may differ across schools of medical thought. Varying terminology should be arranged in groups of synonyms. For example, “intermittent gout, intercritical period” can be clustered with “intercritical gout”. It is important to select an entry that will be used by the expert system as the main term and to identify its synonyms that will redirect the system to the main term. Usually, the most

Table 1. Clinical forms of gout

Classification of clinical phases	Functional classification of joint damage
Acute gouty arthritis	Functional class I
Intermittent gout, intercritical period	Functional class II
Acute intermittent gout. Acute gouty arthritis	Functional class III
Chronic tophaceous gout. Chronic gouty arthritis, intercritical period	Functional class IV
Chronic tophaceous gout. Chronic gouty arthritis, acute period	

commonly used term is assumed to be the main term. All abbreviations of the term are regarded as its synonyms.

4. Extract umbrella terms to arrange revised terminology into groups. For example, "tophus" is an umbrella term for "subcutaneous tophi", "intraosseous tophi", and some other entities.

5. Medicinal drugs should be represented by their active pharmaceutical substances only; it is important to exclude trade names from the nomenclature.

6. Extract quantitative terms. For example, the entry "serum urate" should be annotated with its reference intervals.

Prior to working on the nomenclature, the expert panel enunciated a list of final diagnoses based on their clinical experience and verified literature sources [1, 19]. There were two components to the formulated diagnoses: the clinical stage of gout and the functional class of the affected joint (Table 1).

The analysis of textual sources yielded a nomenclature of 132 main terms and 77 synonyms pertaining to gout diagnosis. This list was revised by the expert panel; the final version of the "diagnostic" nomenclature encompassed 170 main terms and 470 synonyms. For gout therapy, the initial nomenclature compiled from literature sources included 324 main terms and 213 synonyms; its revised version comprised 387 main terms and 515 synonyms.

Some of the terms were present in both nomenclatures; after the nomenclatures were merged, the total number of main terms and their synonyms reached 495 and 679, respectively.

The main terms were broken down in 8 types (Table 2). In the future, this terminology will be used for building an ontology to aid the diagnosis and treatment of gout.

Diagnostic algorithms

Based on Federal Clinical Guidelines [1, 19], diagnostic algorithms for gout were elaborated in a series of steps and revised. Diagnostic algorithms were developed for each of 20 definitions of clinical forms of gout formulated by the expert panel. A total of 40 scenarios were proposed (Table 3) and 52 synonyms were used.

The refined definitions of clinical forms of gout are variants of the final diagnosis that are based on various combinations of the clinical stage of gout and the functional classification of the affected joint [19, 20] (see Table 1). From the diagnostic perspective, both components can be regarded as independent entities. The algorithm for classifying the functional state of the affected joint is fully consistent with the Federal Clinical Guidelines [19].

The algorithm for identifying the clinical stage of gout was based on the 2015 ACR/EULAR international classification criteria [1]. The algorithm consists of 3 steps. The first step is essentially the recognition of acute gout, which involves the analysis of symptoms indicative of inflammation in the ankle, first metatarsophalangeal and mid-foot joints. While refining and revising the nomenclature, the panel expanded the list of symptoms to include knee joint inflammation and peripheral joint inflammation, which refers to the inflammation of 59-61 joints that are at lower risk for being affected by gout. Each feature (symptom) contributes to the final diagnosis and is assigned points. The threshold score for diagnosing gout is ≥ 8 points on the ACR/EULAR scale. However, the following criteria must be taken in account when using the ACR/EULAR classification:

Time-course of the disease

Some features contribute to the diagnosis when used in combination with other features. For example, "time to maximal pain during the attack" < 24 h and "duration of gouty arthritis attack" of 10–14 days jointly score 1 point. The co-occurrence of these symptoms with the feature called "complete resolution of gouty arthritis symptoms" (in 10–14 days) does not affect the total score. Here, an individual feature is not assigned any points. No less significant is the combination of temporal characteristics of the disease with recurrent typical episodes: it increases the total score by 2 points.

Serum urate levels

"Serum urate" can score from –2 to 4 points, depending on its actual concentration in the blood serum. Negative values

Table 2. Types of entities used in the proposed medical nomenclature

№	Type	Description
1	Symptom	Disease elements identified by the clinician by means of various tests. Examples: swelling of the first metatarsophalangeal joint, serum urate level
2	Diagnosis	Clinical diagnosis. Example: acute gouty arthritis
3	Study method	Methods for studying disease elements
3.1	Instrumental method	Instrumental methods for studying disease elements. Example: ultrasound imaging of the joint
3.2	Laboratory method	Laboratory methods for studying disease elements. Example: blood test
3.3	Diagnostic technique	Physical assessment techniques (palpation, percussion, auscultation) and medical history taking. Example: joint mobility assessment
3.4	Medical consultation	Specialist consultations. Example: consultation with a nephrologist
4	Therapy	Treatment methods
4.1	Active substance	Active substances for gout treatment. Example: allopurinol
4.2	Non-pharmacological therapy	Methods of non-pharmacological treatment of gout. Example: Diet № 6

Table 3. Number of diagnostic algorithms for gout; functional classification of joint damage is not included

№	"Diagnosis (functional class of joint damage not included)"	Number of algorithms without FC	Number of algorithms with FC
1	Acute gouty arthritis	2	8
2	Intercritical gout	1	4
3	Acute intermittent gout. Acute gouty arthritis	2	8
4	Chronic tophaceous gout. Chronic gouty arthritis, intercritical phase	3	12
5	Chronic tophaceous gout. Chronic gouty arthritis, acute phase	2	8
	Total	10	40

should also be accounted for when developing rules for a clinical decision support system.

Polarized light microscopy of synovial fluid

According to clinical guidelines, the findings of polarized light microscopy of synovial fluid should be described in terms of 1) presence of monosodium urate crystals in synovial fluid (8 points), 2) absence of monosodium urate crystals in synovial fluid (–2 points) and 3) no test results available (0 points).

Importantly, it is not the binary choice (the presence or absence of a symptom) that should be implemented in a clinical decision support system, but the choice between 3 states: the presence of a symptom, its absence or no data available.

The algorithms, their scores and the logic for score calculation needed for further ontology building were described in MS Excel. Symptoms that could be expressed numerically were represented as a range of possible values and units of measurement consistent with the information in the Units of Measurements reference book [21].

The designed diagnostic algorithms were approved by the expert panel using the comparison method. The experts confirmed that the algorithms were verified and could be used for ontology building.

Algorithms for gout treatment

The process of developing algorithms for gout treatment differed from the process of developing diagnostic algorithms. Treatment algorithms were broken down into the following steps: 1) selecting a general therapeutic strategy; 2) selecting an optimal pharmaceutical substance(s); 3) determining hospitalization criteria.

To provide a clear description of the therapeutic strategy, the panel of experts working in collaboration with the authors of this paper proposed a logical model that comprised 50 different scenarios of treating a gout attack. The algorithms were visualized in MS Visio (Fig. 1), which enabled us to discuss every scenario without going into technicalities. The following steps are included in each scenario:

1) a drug category is chosen (a specific drug will be proposed in the next step); the choice is made between colchicine, NSAIDs, proton pump inhibitors, glucocorticoids, canakinumab, and the combination of these drugs. Due to the specifics of glucocorticoid therapy, glucocorticoid entries are annotated with information about the route of administration (intra-articular, intramuscular, oral);

2) the timeline for medical consultations is specified; consultations are seen as transition points between different treatment scenarios and hospitalization;

3) criteria are set for scenario selection and switching between different scenarios.

Scenarios for gout attack prevention (small doses of colchicine, NSAIDs, glucocorticoids) and serum urate lowering (allopurinol, febuxostat) should be analyzed separately.

In the second step, one or several active pharmaceutical substances are selected as a possible treatment option. These substances will be recommended later, in the form of a regimen that accounts for the drug dose, dosing frequency, route of administration, etc. Each active substance is annotated with a list of contraindications so that dangerous prescriptions could be avoided.

Treatment algorithms were described in MS Excel (Fig. 2). The resultant table contains information returned to the user by the system if the specified criteria are met.

The following rules and limitations were introduced during the development of treatment algorithms for gout patients:

1. Priority should be given to a higher-ranked drug, i.e. the system will recommend the drug if prescription criteria are met and no contraindications are detected.

2. If none of the drugs (active substances) can be prioritized or prescription criteria for these drugs are different, the drugs should be grouped together (see the Group number box in Fig. 2).

3. If several active substances are prescribed, this information will be visually presented to the user as shown in Fig. 3. Example: a combination therapy of one NSAID and one PPI is recommended. Priority is set according to the specified criteria but information is presented to the user in separate blocks.

Thus, the entire process of treatment can have a structured formal representation; at the same time, the rules underlying decision making are available to the user and can be analyzed.

The proposed treatment algorithms were compared, verified and recommended for further ontology building by the panel of experts.

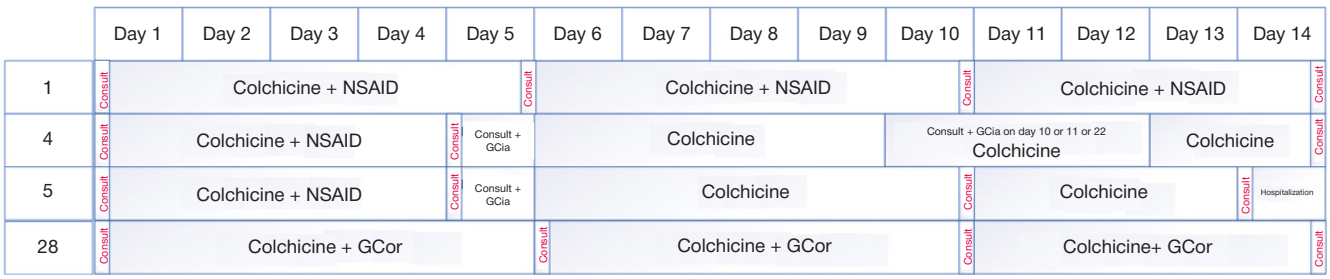

Fig. 1. Visual representation of a teratment scenario. GCia — intra-articular glucocorticoid, GCor — oral glucocorticoid

	Active substance	Dose	Unit of measurement	Dosing frequency	Unit of measurement for dosing frequency	Therapy duration	Unit of time	Route of administration
1	Naproxen	275	mg	3	Once a day	8	Day	Oral
2	Nimesulide	100	mg	2	Once a day	8	Day	Oral
3	Diclofenac	50	mg	3	Once a day	8	Day	Oral
4	Etoricoxib	120	mg	1	Once a day	8	Day	Oral
5	Naproxen	550	mg	2	Once a day	8	Day	Oral
6	Nimesulide	100	mg	2	Once a day	8	Day	Oral
7	Diclofenac	50	mg	3	Once a day	8	Day	Oral
8	Etoricoxib	120	mg	1	Once a day	8	Day	Oral
9	Celecoxib	400	mg	1	Once a day	8	Day	Oral
10	Aceclofenac	200	mg	1	Once a day	8	Day	Oral
11	Tenoxicam	20	mg	3	Once a day	8	Day	Intramuscular
12	Ketoprofen	100	mg	2	Once a day	8	Day	Intramuscular
13	Dexketoprofen	25	mg	3	Once a day	8	Day	Oral
14	Dexketoprofen	50	mg	3	Once a day	8	Day	Intravenous
15	Indomethacin	25	mg	3	Once a day	8	Day	Oral
16	Ibuprofen	400	mg	3	Once a day	8	Day	Oral
17	Piroxicam	20	mg	1	Once a day	8	Day	Oral
18	Lornoxicam	8	mg	2	Once a day	8	Day	Oral
19	Etodolac	400	mg	3	Once a day	8	Day	Oral

	Active substance	Dose	Unit of measurement	Dosage form	Timing with meals	Prescription criteria	Special notes	Group number
1	Naproxen	275	mg	Tablets	Irrespective of meals	Age (>65 years); Colchicine; Duration of acute gouty arthritis (>3 days)		1
2	Nimesulide	100	mg	Tablets	Irrespective of meals	Age (>65 years); Colchicine; Duration of acute gouty arthritis (>3 days)		1
3	Diclofenac	50	mg	Tablets	Irrespective of meals	Age (>65 years); Colchicine; Duration of acute gouty arthritis (>3 days)		1
4	Etoricoxib	120	mg	Tablets	Irrespective of meals	Age (>65 years); Colchicine; Duration of acute gouty arthritis (>3 days)		1
5	Naproxen	550	mg	Tablets	Irrespective of meals	Colchicine; Duration of acute gouty arthritis (>3 days)		2
6	Nimesulide	100	mg	Tablets	Irrespective of meals	Colchicine; Duration of acute gouty arthritis (>3 days)		2
7	Diclofenac	50	mg	Tablets	Irrespective of meals	Colchicine; Duration of acute gouty arthritis (>3 days)		2
8	Etoricoxib	120	mg	Tablets	Irrespective of meals	Colchicine; Duration of acute gouty arthritis (>3 days)		2
9	Celecoxib	400	mg	Capsules	Irrespective of meals	Colchicine; Duration of acute gouty arthritis (>3 days)		3
10	Aceclofenac	200	mg	Prolonged-release tablets	With meals	Colchicine; Duration of acute gouty arthritis >3 days)		3
11	Tenoxicam	20	mg	For injection, lyophilized powder for intramuscular use	Irrespective of meals	Colchicine; Duration of acute gouty arthritis (>3 days)		4
12	Ketoprofen	100	mg	For injection, for intramuscular use	Irrespective of meals	Colchicine; Duration of acute gouty arthritis (>3 days)		4
13	Dexketoprofen	25	mg	Granules, for solution	Irrespective of meals	Colchicine; Duration of acute gouty arthritis (>3 days)		4
14	Dexketoprofen	50	mg	For injection, for intravenous use	Irrespective of meals	Colchicine; Duration of acute gouty arthritis (>3 days)		4
15	Indomethacin	25	mg	Tablets	Irrespective of meals	Colchicine; Duration of acute gouty arthritis (>3 days)		4
16	Ibuprofen	400	mg	Tablets	With meals	Colchicine; Duration of acute gouty arthritis (>3 days)		4
17	Piroxicam	20	mg	Tablets	With meals	Colchicine; Duration of acute gouty arthritis (>3 days)		4
18	Lornoxicam	8	mg	Tablets	Irrespective of meals	Colchicine; Duration of acute gouty arthritis (>3 days)		4
19	Etodolac	400	mg	Tablets	Irrespective of meals	Colchicine; Duration of acute gouty arthritis (>3 days)		4

Fig. 2. An example of treatment algorithm (a nonsteroidal anti-inflammatory drug)

			Active substance	One-time dose	Unit of measurement for single dose
Combination therapy with nonsteroidal anti-inflammatory drugs and proton pump inhibitors is recommended	Nonsteroidal anti-inflammatory drugs	1	Celecoxib	400	mg
		2	Etoricoxib	120	mg
		3	Nimesulide	100	mg
	Proton pump inhibitors	1	Pantoprazole	40	mg
		2	Omeprazole	20	mg
		3	Rabeprazole	20	mg
		4	Esomeprazole	40	mg
		5	Dexlansoprazole	30	mg
		6	Lansoprazole	15	mg

Fig. 3. Visual representation of combination therapy with active substances

DISCUSSION

The proposed verified nomenclature of medical entities pertaining to the diagnosis and treatment of gout has laid the foundation for the future ontology. The rigorous classification scheme and the generalization of entities suggest that the conceptual model, which is currently in development, has a specification: each entity included in the model has an explicitly described role. Categorizing the entities into the groups of main terms and their synonyms is important for ontology building because it allows using terminologies from different schools of medical thought and expanding the existing list of terms. Thus, the principle of a shared conceptual model is implemented. Because clinical diagnoses had unambiguous definitions, we were able to proceed to the development of treatment algorithms for gout patients.

The proposed verified diagnostic algorithms will be later used to build the diagnostic domain of the future ontology. The identified patterns (diagnostic stages, specific work of logic with individual entities (or their groups) that contribute to the diagnosis of gout, the need to use 3 states for some entities that explicitly affect the diagnosis) will underpin the structure of the future ontology, help to fill it with data and be used to elaborate algorithms for the expert system. Unambiguous definition of units of measurement for all quantitative symptoms also suggest that our conceptual model has a specification.

The proposed verified treatment algorithms will be used to build the treatment domain of the future ontology, fill it with data and develop an expert system for the optimization of gout treatment regimens and patient monitoring.

The algorithms proposed in this paper are described in MS Excel. This means that the accumulated knowledge now has a formal representation and can be transferred to an ontology by means of special software tools. On the other hand, MS Excel tools can be used by experts to further expand the ontology.

We were unable to compare our medical nomenclature of gout-related terms with similar informational objects because currently existing expert systems either are based on a different approach [22] or do not provide data for comparison [23]. On the other hand, there is a wealth of studies that utilize similar methods of knowledge extraction, structuring and formalization but pertain to other medical fields, like angina pectoris [24], intestinal bleeding [25], etc. However, it is impossible to objectively compare the results of those studies with the results of our work.

CONCLUSIONS

We have developed a medical nomenclature of terms pertaining to the diagnosis and treatment of gout using literature sources and expert opinions. The diagnostic domain includes 179 main terms and 470 synonyms, whereas the treatment domain comprises 387 main terms and 515 synonyms. We have also designed formal diagnostic and treatment algorithms for gout patients (40 and 50 scenarios, respectively) that will be instrumental in building a clinical decision support system suitable for use in an outpatient setting.

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BILATERAL LACERTUS FIBROSUS ENTRAPMENT OF MEDIAN NERVE AT THE ELBOW (LACERTUS SYNDROME)

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Lacertus fibrosus entrapment of the median nerve at the elbow, or Lacertus syndrome, is a rare pathology, which is difficult to detect by instrumental examination methods. Literary sources provide no information on the syndrome prevalence, as well as on precise diagnosis and treatment algorithms, which makes it difficult to establish the diagnosis and appoint the effective treatment. Lacertus syndrome is characterized by positive scratch collapse test; differential diagnosis is required to distinguish it from carpal tunnel syndrome. Clinical case of bilateral lacertus fibrosus entrapment of the median nerve at the elbow is reported, Lacertus syndrome diagnosis criteria and surgical treatment method are set out. The results of the surgical tactics used are as follows: pain, paresthesia and numbness in the innervation field of the median nerve have vanished, the muscle strength has been restored to level M5 in accordance with MRC muscle strength scale. These results demonstrate high efficiency of the proposed lacertus syndrome surgical treatment.

Keywords: median nerve entrapment, Lacertus syndrome, bicipital aponeurosis, lacertus fibrosus

Author contribution: Ishikhov IM — study concept, literature analysis, data interpretation, manuscript writing; Kolomiets KV — literature analysis, data interpretation; Gamidov FM — patient's treatment and follow-up, manuscript writing; Gantsgorn EV — data interpretation, manuscript editing.

Compliance with ethical standards: the informed consent for surgery and personal data processing was obtained from the patient.

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БИЛАТЕРАЛЬНАЯ КОМПРЕССИЯ СРЕДИННОГО НЕРВА АПОНЕВРОЗОМ ДВУГЛАВОЙ МЫШЦЫ ПЛЕЧА НА УРОВНЕ ЛОКТЕВОГО СУСТАВА (ЛАЦЕРТУС-СИНДРОМ)

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Компрессия срединного нерва апоневрозом двуглавой мышцы плеча (*lacertus fibrosus*) на уровне локтевого сустава, или Лацертус-синдром, — редкая патология, которую сложно обнаружить с помощью инструментальных методов исследования. В литературе отсутствуют сведения о распространенности данного синдрома, четкие алгоритмы его диагностики и лечения, что затрудняет постановку диагноза и назначение эффективного лечения. Лацертус-синдром сопровождается положительным *scratch collapse test* и требует дифференциальной диагностики с синдромом карпального канала. Представлен клинический случай пациента с билатеральной компрессией срединного нерва апоневрозом двуглавой мышцы плеча на уровне локтевого сустава, изложены критерии диагностики Лацертус-синдрома, способ оперативного лечения. Результаты использованной хирургической тактики: исчезновение боли, парестезий и онемения в зоне иннервации срединного нерва, восстановление силы мышц до уровня М5 по Шкале оценки мышечной силы MRC — свидетельствуют о высокой эффективности предлагаемого хирургического лечения Лацертус-синдрома.

Ключевые слова: компрессия срединного нерва, Лацертус-синдром, апоневроз двуглавой мышцы плеча, *lacertus fibrosus*

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Соблюдение этических стандартов: от пациента получено добровольное информированное согласие на оперативное лечение и обработку персональных данных.

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Traditional wisdom is that proximal median nerve entrapment (PMNE) at the level of the elbow is a rare diagnosis, since it is difficult to detect using such electrophysiological techniques as electroneuromyography (ENMG) [1].

In foreign literature the disorder is referred to as Lacertus syndrome (LS). The detailed description for this syndrome was first provided in 2013 by Elisabet Hagert (Sweden), who also proposed a method for LS diagnosis based on the following clinical manifestations: weakness in muscles innervated by the median nerve distal to the lacertus fibrosus (LF), i.e. in flexor hallucis longus, flexor digitorum profundus II, and flexor carpi radialis; pain upon pressure over the median nerve at the level of the LF; positive scratch collapse test [1]. Later, this criterion contributed to a more frequent LS detection in patients of different age groups, and to more accurate differential diagnosis to distinguish LS from carpal tunnel syndrome, which often has similar clinical manifestations.

However, there are still just a few papers on the issue in medical literature, which hinders wide dissemination of knowledge on differential diagnosis and treatment of LS among doctors of different specializations, and makes it almost impossible to assess the prevalence of LS in certain countries. To date, no publications on the lacertus fibrosus entrapment of the median nerve at the elbow can be found in domestic sources, which accounts for lack of favorable outcome in treatment of patients with such pathology.

The clinical features of the syndrome are relatively nonspecific (episodes of numbness, paresthesia in the innervation field of the median nerve, pain in the area of lacertus fibrosus, loss of strength in flexor hallucis longus, flexor digitorum profundus II, and flexor carpi radialis), the disorder shares some similarities with carpal tunnel syndrome [2, 3]. Therefore, the patients may show no treatment effect for a long time.

The paper reports a clinical case of LS; diagnostic approach and tactics of surgical treatment for patients with clinical signs of median nerve entrapment are being proposed.

Clinical case

Patient A. aged 32, was admitted to Clinical Hospital "Railways-Medicine", Rostov-on-Don, because of the decreased first three fingers flexion strength, numbness, creeping sensation in the palmar surface of the first three fingers on both hands, which intensified during computer work and any physical work involving hands. The patient denied any injuries to his left and right upper limbs.

According to the patient, numbness and weakness in the fingers had emerged for the first time 6 years before in the right hand. Then similar symptoms emerged in the left hand. Loss of sensitivity in the first three fingers, as well as rapid loss of the thumb and index finger strength were the first symptoms of the disorder, which occurred while playing games using joystick forcing the patient to quit the game. These symptoms vanished after a few minutes of rest, but kept coming up each time he played games. Subsequently, the symptoms progressed: they occurred during computer work and guitar playing. After neurological assessment, instrumental examination was recommended: magnetic resonance imaging (MRI) of the cervical spine, electroneuromyography (ENMG) of the upper limbs, ultrasonography along the entire length of the median nerve. MRI revealed no degenerative/dystrophic changes in the cervical spine; ENMG detected no upper limb peripheral nerve pathology; ultrasonography revealed sonographic signs of bilateral carpal tunnel syndrome, as well as sonographic signs of flexor tendinitis. The diagnosis of bilateral carpal tunnel syndrome was established based on the examination results. Conservative treatment consisting of non-steroidal anti-inflammatory drugs (NSAIDs), anticonvulsants, B vitamins, massage of the upper limbs, physiotherapy (phonophoresis with hydrocortisone applied over the wrist joints № 10, exercise therapy, acupuncture) was prescribed. During treatment the patient noted that only regular exercise therapy could produce some improvement. Lack of significant improvement made the patient to contact hand surgeon.

Examination of the left and right upper limbs revealed no visible changes. Palpation elicited pain in the area of the LF ligament. During hand pronation, paresthesias in the first three fingers along the palmar surface were observed. Resistive testing revealed loss of strength in flexor hallucis longus, flexor digitorum profundus II, and flexor carpi radialis, which corresponded to M3 criterion ("The muscle can move the joint it crosses through a full range of motion against the gravity but without applying any resistance") of the Medical Research Council Weakness Scale (MRC) [4]. Scratch collapse test was positive above the LF ligament. The testing method is as follows [5]: the patient sits down facing the surgeon with elbows flexed and forearms extended, his wrists in a neutral position. *Step 1*: the patient resists bilateral shoulder adduction (internal rotation of the forearms) performed by the surgeon. *Step 2*: the surgeon "scratches" the skin or runs his fingers along the projection of entrapped median nerve at the level of lacertus fibrosus. *Step 3*: the surgeon immediately repeats Step 1. A positive response to scratch collapse test is a temporary loss of external resistance. Tinel's sign, Durkan's compression test and Phalen's test were negative, which made it possible to exclude carpal tunnel syndrome. Active movements of upper limb joints were performed over full range of motion. No vascular dysfunction was observed at the time of examination. The following clinical diagnosis was established: G56.1 — Other lesions of the median nerve.

Surgical treatment was recommended: ligamentotomy of the LF ligament, decompression of median nerve in the left and right upper limbs.

Surgical technique

At the first stage of the procedure it was necessary to differentiate LF. For that, we identified the distal biceps tendon and found a dense ligament woven into the deep fascia of the forearm to the medial side of the distal biceps tendon by

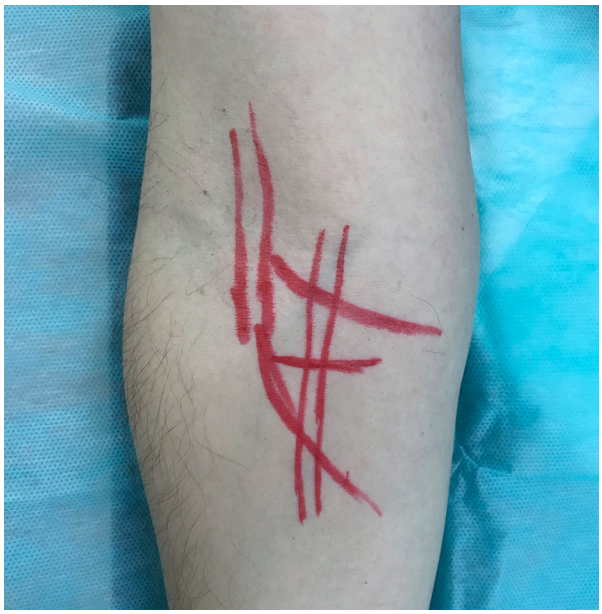

Fig. 1. Preoperative markup

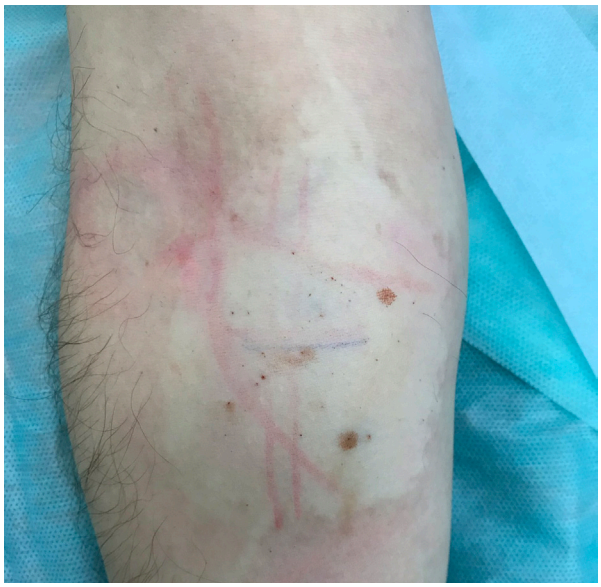

Fig. 2. WALANT (Wide-Awake Local Anesthesia No Tourniquet) local infiltration anaesthesia

palpation. Incision was planned over the ligament. Preoperative markup is presented in Fig. 1.

The surgical procedure was performed in an outpatient setting under WALANT local infiltration anesthesia (Fig. 2).

For that the patient was anesthetized 20 min preoperatively using 20 mL of 1% lidocaine (10 mg/mL) with 0.2 mL epinephrine (5 μg/mL) and 2–3 mL of sodium bicarbonate buffer (50 mg/mL) used to stabilize the lidocaine pH.

A 2–3 cm transverse incision was placed 2–3 cm aside from the flexion crease of the cubital fossa, 1–2 cm medial of the biceps tendon (Fig. 3). The skin incision was placed parallel to flexion elbow crease, which ensured good postoperative aesthetic appearance. Careful dissection was made subcutaneously to identify the medial antebrachial cutaneous nerve, which can occasionally be found in the zone of access before it reaches the pronator teres fascia. The pronator teres fascia was incised and slightly retracted medially. LF was easily visualized in the center (Fig. 4) and dissected in the transverse direction of fiber orientation. After dissection of LF the median nerve was identified, sometimes housed in the pronator teres muscle belly (Fig. 5).

The resistive testing was performed prior to skin closure, since the muscle strength is usually restored immediately after the release of the nerve [6]. Active flexion with overcoming resistance of flexor hallucis longus, flexor digitorum profundus II, and flexor carpi radialis had fully recovered. After hemostatic procedures, subcuticular suturing was performed with 4–0 monofilament suture (Fig. 6). Sterile dressing was applied. Immobilization was not required.

The patient returned to normal routine 2 days after surgery. He was advised to avoid lifting of loads heavier than 1 kg during the first 2 weeks, as well as to avoid physical exercise. Heavier loads and physical work were permitted 4 weeks after surgery.

Discussion

Clinical manifestations of LS vanished intraoperatively after the median nerve decompression. Muscle strength in flexor hallucis longus, flexor digitorum profundus II, and flexor carpi radialis recovered completely, which fulfilled the M5 criterion ("The muscle can move the joint it crosses through a full range of motion, against the gravity as well as against full resistance applied by the examiner — Normal power") of the MRC scale.

A month after the procedure the scratch collapse test became negative; numbness, and creeping sensation in the palmar surface of the left and right hand first three fingers resolved completely. During the postoperative period the patient experienced two episodes of short-term dull pain in muscles of forearm and palm, and voiced no other complaints. The scar is pink and painless, with no adhesions.

Two months after surgery on his right hand the patient underwent similar surgical procedure on his left hand. After 3 months the patient returned to normal routine and got a job (undiagnosed LS and ineffective conservative treatment made him unable to work for almost 5 years).

Similar favorable results of the applied LS surgical treatment method (particularly, in terms of muscle strength recovery) have been reported in the paper by E. Hagert (2020): after surgery the patients, advised to limit physical activities for some time, return to light duties within 1–2 days [6].

Conclusion

The clinical case reported demonstrates the importance of raising awareness about clinical manifestations, diagnosis

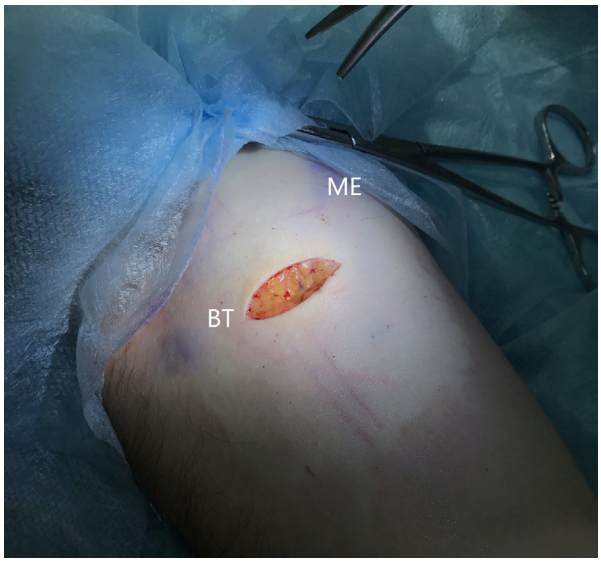

Fig. 3. Incision. BT — *biceps tendon*; ME — *medial epicondyle*

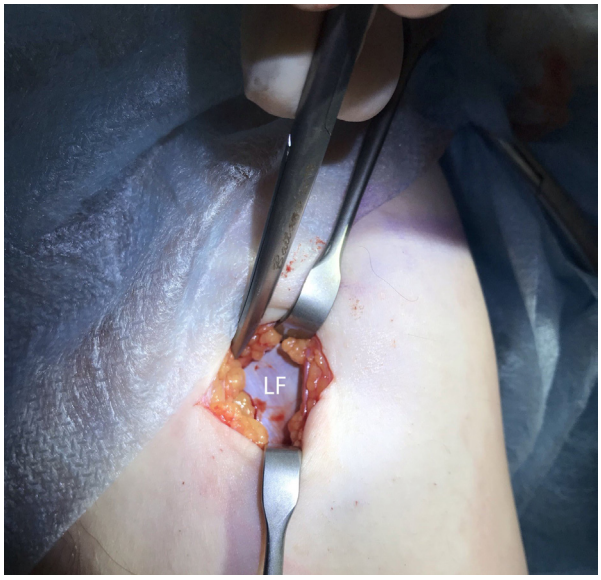

Fig. 4. *Lacertus fibrosus* (LF) ligament is carefully isolated and divided in its entire length

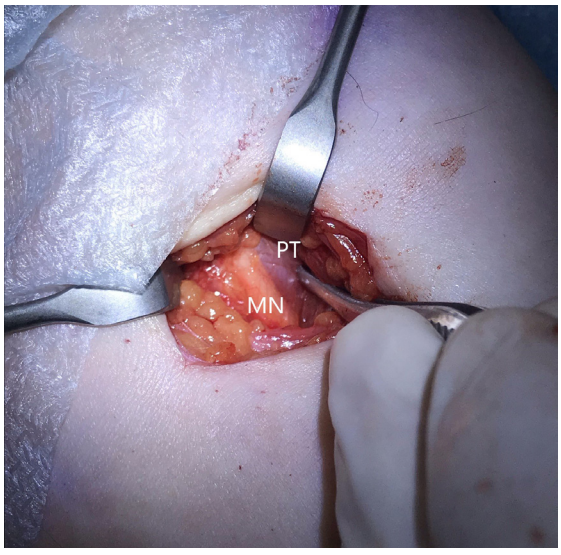

Fig. 5. Median nerve is easily seen after LF division. MN — *median nerve*; PT — *pronator teres*

methods and surgical treatment of LS. In this example the diagnosis was established based on the following clinical signs combination: weakness in muscles innervated by median nerve distal to LF (flexor hallucis longus, flexor digitorum profundus II, and flexor carpi radialis); pain elicited by pressure on the median nerve at the level of LF; positive scratch collapse test and negative compression tests. We believe that it would be appropriate to recommend taking into account the stated diagnosis criteria when examining patients with clinical manifestations of median nerve entrapment. Correct differential diagnosis of LS makes it possible both to avoid appointment of unnecessary conservative therapy (particularly, of NSAIDs and anticonvulsants), and to provide timely delivery of adequate surgical care allowing to ensure patients' recovery and the quality of life improvement as quickly as possible.

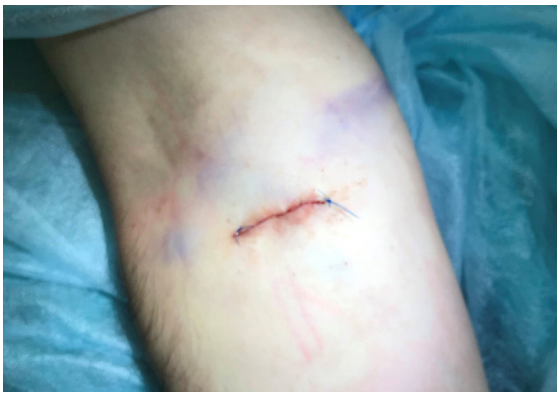

Fig. 6. Postoperative wound

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EFFECTIVENESS OF POST-STROKE SOCIAL REHABILITATION IN PATIENTS WITH MODERATE IMPAIRMENTS

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The importance of post-stroke rehabilitation cannot be overestimated. The aim of this study was to assess the effectiveness of a standardized post-stroke rehabilitation program for patients with moderate impairments. A total of 122 stroke survivors participated in the study. Group 1 (the comparison group) consisted of 59 patients undergoing standard rehabilitation. Group 2 (the main group) consisted of 63 patients participating in a specially designed social rehabilitation program. The functional, cognitive, psychological and emotional states and the level of social activity were monitored for 6 months. Group 2 demonstrated more pronounced improvement than group 1 after 6 months of follow-up, confirmed by higher Rivermead ($p = 0.011$) and SS-QOL ($p < 0.05$) scores and lower DASH and Beck scores ($p = 0.015$ and $p < 0.001$, respectively). In both groups, MMSE (cognitive function) scores slightly increased, but the differences between the groups were insignificant. The proposed post-stroke rehabilitation program is effective in helping patients regain their independence and improve social adaptation. The scales and scores used in the study, especially DASH and SS-QOL, are sensitive to changes in the functional state of stroke survivors with moderate impairments.

Keywords: stroke, rehabilitation, social functioning assessment, cognitive status, physical activity, neurological status, quality of life

Author contributions: Khramov VV planned the study and wrote the manuscript; Kogaeva KP planned and conducted the study and wrote the manuscript; Arkhipova LU reviewed the literature and interpreted the obtained data; Alekseeva VO, Lukyanova MI performed statistical analysis.

Compliance with ethical standards: the study was approved by the Ethics Committee of Saratov State Medical University (Protocol № 1 dated September 1, 2020). All participants gave informed consent to participate in the study.

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ЭФФЕКТИВНОСТЬ МЕДИКО-СОЦИАЛЬНОЙ РЕАБИЛИТАЦИИ ПОСТИНСУЛЬТНЫХ ПАЦИЕНТОВ С НАРУШЕНИЯМИ СРЕДНЕЙ СТЕПЕНИ ТЯЖЕСТИ

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На сегодняшний день остается несомненной актуальность реабилитационных мероприятий у пациентов, перенесших острые нарушения мозгового кровообращения. Целью работы было оценить эффективность стандартизированной медико-социальной программы реабилитации постинсультных пациентов с нарушениями средней степени тяжести. В исследовании участвовало две группы пациентов (всего 122 человека), перенесших инсульт: 59 пациентам группы 1 (сравнения) проводили стандартную программу реабилитации; 63 пациентам группы 2 (основной) — разработанную программу социальной реабилитации. Изучено динамическое состояние пациентов по показателям шкал оценки функционального, когнитивного, психоэмоционального статуса и социальной активности в течение полугода. У пациентов группы 2 через 6 месяцев после начала наблюдения отмечена более выраженная (относительно группы сравнения) динамика изучаемых показателей, что подтвердили статистически значимо более высокие значения показателей шкал Ривермид ($p = 0,011$) и SS-QOL ($p < 0,05$) и более низкие значения показателей шкал DASH и шкалы Бека ($p = 0,015$ и $p < 0,001$ соответственно). В обеих группах выявлено некоторое увеличение показателей шкалы оценки когнитивных нарушений MMSE, однако значимых межгрупповых различий при этом не отмечено. Разработанная программа медицинской реабилитации пациентов, перенесших ОНМК, эффективна для восстановления навыков самообслуживания, восстановления социально значимых навыков, таких как социально-бытовая адаптация. Использованные в работе шкалы и опросники чувствительны к изменениям функционального статуса пациентов с постинсультными нарушениями средней степени тяжести, в первую очередь — шкалы DASH и SS-QOL.

Ключевые слова: инсульт, реабилитация, социальная оценка, когнитивный статус, физическая активность, неврологический статус, качество жизни

Вклад авторов: В. В. Храмов — планирование исследования, подготовка и редактирование рукописи; К. П. Когаева — планирование и проведение научных исследований, подготовка и редактирование рукописи; Л. Ю. Архипова — обзор литературы, интерпретация данных; В. О. Алексеева, М. И. Лукьянова — статистический анализ.

Соблюдение этических стандартов: исследование одобрено этическим комитетом Саратовского ГМУ имени В. И. Разумовского (протокол № 1 от 1 сентября 2020 г.); все участники подписали добровольное информированное согласие на участие в исследовании.

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Stroke is among the leading causes of morbidity and long-term disability in high-income countries. In Russia, the incidence of stroke is 3–4 cases per 1,000 population. Survival has improved to 85% in the past few years; currently, there are over 1,000,000 stroke survivors living in Russia, of whom 80 percent are disabled. So, post-stroke rehabilitation is becoming increasingly important for economy and society [1–6].

Stroke is a life-changing event both at the individual and community levels. Its sequelae prevent the patient from returning to work, lead to social isolation and are a heavy burden for economy [3, 5, 7, 8].

It is known that stroke can have a long-lasting impact on personal, familial and social well-being that persists after primary rehabilitation. Patients and their caregivers need access to rehabilitation resources and services [9–14]. Medical and social care is provided by healthcare specialists, social workers and volunteers [7, 8]. Highly-skilled medical professionals provide effective care in the early rehabilitation period. Then,

other rehabilitation specialists step in to assist patients in social adaptation and relearning self-care skills.

What is known about the needs of stroke survivors and their caregivers is usually known from clinical data and observational studies which analyze a number of parameters using mostly non-standardized methods. That said, such data are sufficient to highlight some important problems, including social and emotional consequences of stroke that persist for many years after the initial event [15–20].

Considering the recent initiatives of public health agencies aimed at fighting the effects of stroke on individual and public health, it would be interesting to analyze the outcomes of social rehabilitation programs for stroke survivors through the medical and social lens and to estimate the sensitivity of the methods used to assess the functional state of stroke patients.

The aim of this study was to demonstrate the effectiveness of standardized post-stroke rehabilitation for patients with moderate impairments.

METHODS

This prospective randomized controlled parallel-group study was conducted at Kamyshin City Hospital № 1, Volgograd region, between 2016 and 2020. The study enrolled 122 stroke survivors with moderate impairments: 71 women (58.2%) and 51 men (41.8%). The mean age of the participants was 66.5 ± 12.8 years.

The following inclusion criteria were applied: both sexes, age between 18 and 85 years, moderate stroke within 6 previous months. Patients with severe stroke, brain tumors, somatic pathology, or psychiatric disorders were excluded from the study.

The patients were divided into 2 groups based on the type of rehabilitation program:

– group 1 (the comparison group) consisted of 59 patients undergoing standard rehabilitation;

– group 2 (the main group) consisted of 63 patients participating in the rehabilitation program proposed by the authors of this paper.

Patients included in the study were moderate stroke survivors in the late rehabilitation period. The average NIHSS score was 11.75 points. During the study, both groups received 10 sessions of physical exercise, 10 sessions of massage, 10 sessions of physiotherapy, 2 sessions of occupational therapy, and 1 session of social adaptation. Additionally, the main group received 5 sessions of occupational therapy, 5 sessions of social adaptation and 5 sessions of self-care skills.

Progress was evaluated using assessment scales for measuring functional, cognitive, psychoemotional status and social activity. Assessments were made before rehabilitation, after discharge from the hospital, i.e. 12–14 days after admission, and then at 1,3 and 6 months.

Before rehabilitation, all patients underwent a medical checkup and a neurological examination. The following assessment tools were used in the study: the Barthel Index, the Rivermead Mobility Index, the DASH questionnaire, the Beck Depression Inventory, the MMSE questionnaire, and the SS-QoL score (Stroke Specific Quality of Life).

Social and medical rehabilitation of stroke survivors

For the purpose of this study, we designed a social rehabilitation program for stroke survivors. Unlike standard rehabilitation, the amount and type of interventions included in our program varied depending on the stroke sequelae experienced by the patient. The program was designed to fulfill the needs of stroke survivors with moderate impairments. The rehabilitation plan included social adaptation sessions conducted once a month, followed by the analysis of goal achievements. During the session, the patients were tested using the assessment tools listed above. Once every 2 weeks, we called the patients on the phone to check their adherence to the program and modify the tasks if necessary.

Each patient received a detailed written description of the rehabilitation program, with a daily planner for ticking the tasks performed during the day. The following skills were trained: using the bathroom and doing morning hygiene (independently, daily), taking a bath with minimal assistance, getting dressed with minimal assistance, doing up the buttons, tying shoelaces (independently). As part of their rehabilitation, the patients were asked to do simple cooking (make a sandwich or fried eggs) or try more challenging dishes (salad, soup) and do the dishwashing.

Physical activities included in the rehabilitation program were as follows: indoor walking over 200 m, going up and down the stairs, taking a walk outside, doing easy chores (bed making, dusting) or more difficult chores (mopping), gardening,

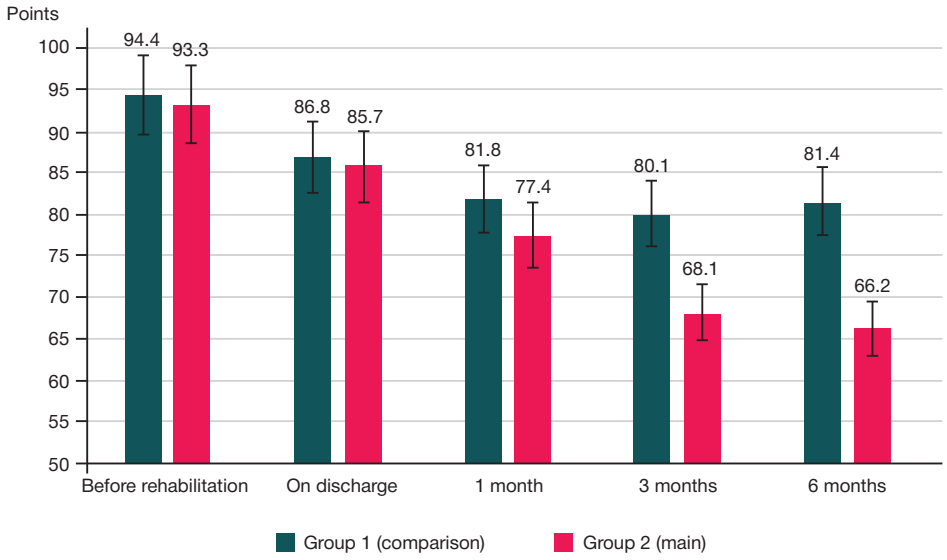

Fig. 1. Dynamics of the Barthel Index, M ± *m*

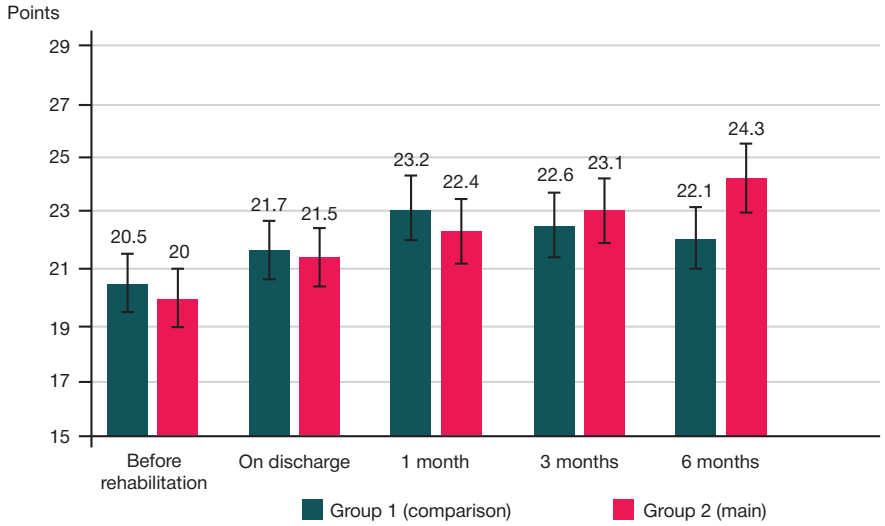

Fig. 2. Dynamics of the Rivermead Mobility Index, M ± *m*

taking care of indoor plants. Social activities included in the program were as follows: visiting family and friends, going to the cinema or theater, and hobbies (drawing, playing chess). We called the patients twice a month to evaluate their progress.

Statistical analysis was carried out in STATISTICA 10 (Statsoft; USA). Mean values and the standard error of the mean were calculated for quantitative parameters. Intergroup comparisons were done using the nonparametric Mann-Whitney *U* test, accounting for the specific character of the analyzed parameters. Differences were considered significant at $p > 0.05$.

RESULTS

The Barthel Index (independence) measured immediately after hospital treatment was higher than on admission. However, there were no significant differences between the groups: 72.7 ± 4.1 vs 72.1 ± 5.5 points for groups 1 and 2, respectively. BI dynamics were positive in both groups after discharge home and at 1, 3, and 6 months, with no significant difference between the groups (Fig. 1). Before rehabilitation, the NIHSS score was 11.4 ± 4.0 in group 1 and 12.1 ± 5.0 points in group 2, with no significant difference between the groups.

Three months after the rehabilitation program was commenced, the Rivermead Mobility Index increased to 8.4 ± 0.4 in the main group ($p = 0.011$); at 6 months, it was 8.4 ± 0.5 (Fig. 2). At both time points, the main group scored significantly higher than the comparison group ($p < 0.05$).

At 3 months, the DASH score declined to 68.1 ± 2.9 points in the main group and was statistically lower ($p < 0.001$) than in the comparison group (80.1 ± 5.2). At 6 months, the situation was the same: 81.4 ± 6.4 points in group 1 vs 66.2 ± 3.1 points in group 2 ($p = 0.015$) (Fig. 3).

In the comparison group, the Beck Depression Inventory score declined to 15.3 ± 1.8 points at 3 months. The score was significantly lower in the main group ($p = 0.008$), equaling 12.7 ± 1.6 points. At 6 months, the situation was the same: group 1 scored 15.8 ± 1.4 points, group 2 scored 13.1 ± 0.9 points ($p < 0.001$) (Fig. 4).

Before rehabilitation, the total MMSE (cognition) score was 20.5 ± 1.5 points in group 1 vs 20.0 ± 1.8 points in group 2. During the follow-up period, this parameter was slightly increasing slightly, with no significant difference between the groups (Fig. 5).

Before rehabilitation, both groups were comparable in terms of all SS-QoL (quality of life) domain scores (Table). At 6 months, the main group scored higher on most domains than the comparison group. For example, group 1 scored an average of 2.60 ± 0.17 points on the "energy" domain, whereas group 2 scored 3.07 ± 0.15 points, which was a significantly higher value ($p < 0.05$). The main group scored significantly

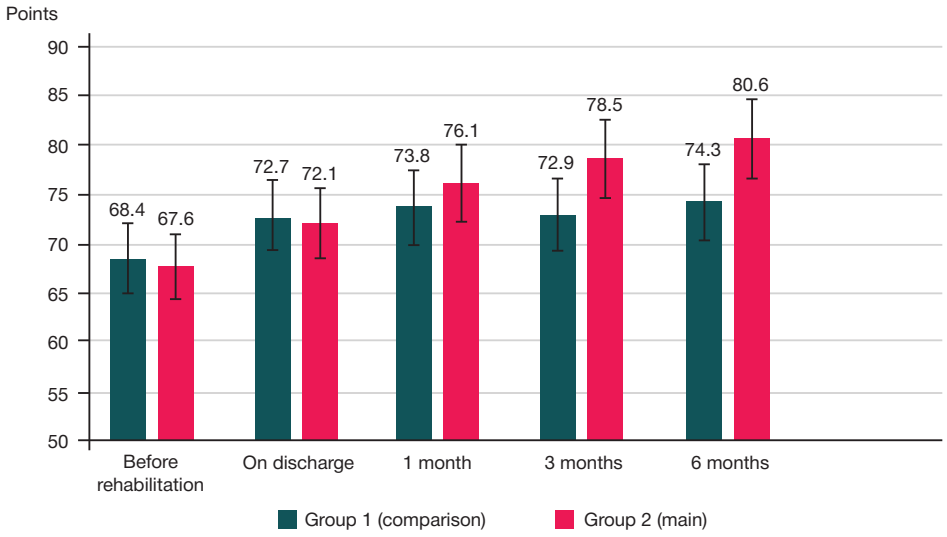

Fig. 3. Dynamics of DASH scores, M ± *m*

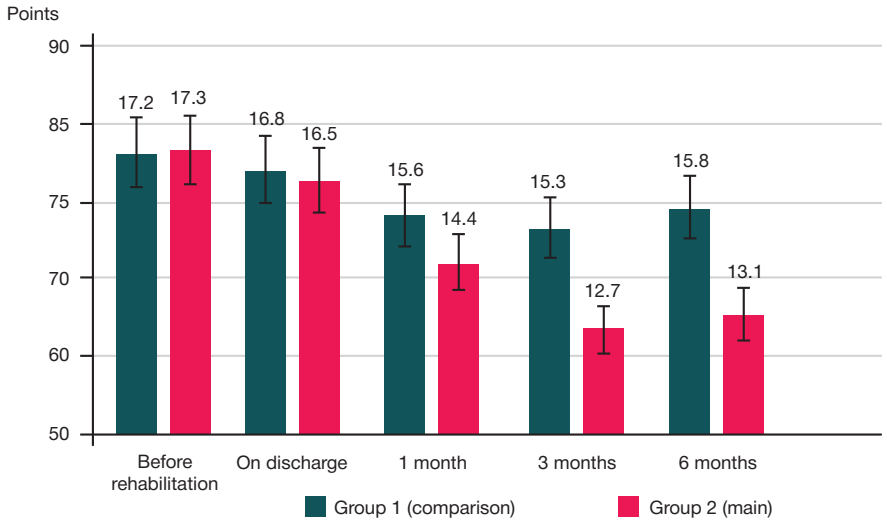

Fig. 4. Dynamics of Beck Depression Inventory scores, M ± *m*

higher on the "family roles" domain than the comparison group, but the "language" score did not differ significantly between the groups. The "mobility" score was significantly higher in group 2 than in group 1 (4.31 ± 0.29 vs 2.92 ± 0.37 points; $p < 0.05$).

The following scores were significantly higher in the main group than in the comparison group ($p < 0.05$): mood (4.34 ± 0.41 vs 2.85 ± 0.28), self-care (4.70 ± 0.19 vs 3.82 ± 0.20), social roles (3.82 ± 0.25 vs 2.74 ± 0.17), work, productivity (3.20 ± 0.28 vs 2.41 ± 0.15), upper extremity function (4.03 ± 0.19 vs 4.74 ± 0.21).

Vision, thinking and personality scores did not change in comparison with the baseline values in both groups.

The analysis shows that the proposed rehabilitation program was effective in helping stroke survivors recover important social skills: families started paying more attention to their stroke-affected members, patients became less dependent on their families. Repetitive self-care training makes the patient more active, reduces functional dependency on family members, and restores the patient's role in the family. Regular meetups with friends and social activities improve social integration. As part of the rehabilitation program, we spoke with our patients on the phone once every two weeks over the course of 6 months and had social adaptation sessions once a month, which allowed us to control adherence to the program and task performance (going for a walk, meeting friends, gardening, hobbies). Regular control and active involvement of relatives in rehabilitation improved social activity of the patients indicated by increased SS-QoL scores.

At 6 months, the dynamics of the studied parameters were more pronounced in the main group than in the comparison group, as seen from the significantly higher Rivermead Mobility Index, higher SS-QoL scores and lower DASH and Beck scores.

DISCUSSION

Today, most experts hold the opinion that recovery of functions affected by the stroke, adaptation to the loss of such functions and social integration should be a top-priority in the rehabilitation of stroke survivors [20–23]. Therefore, it is essential to understand the needs of stroke survivors and challenges they face in order to develop effective approaches involving high-quality medical care and social work services [24–27]. When evaluating the quality of care, it is important to receive a feedback from the patient and their caregiver about the quality of care and to clarify what could be improved.

Our findings are consistent with the results of other studies reporting the mitigating effect of social work services on stress levels and the consequences of stress in stroke

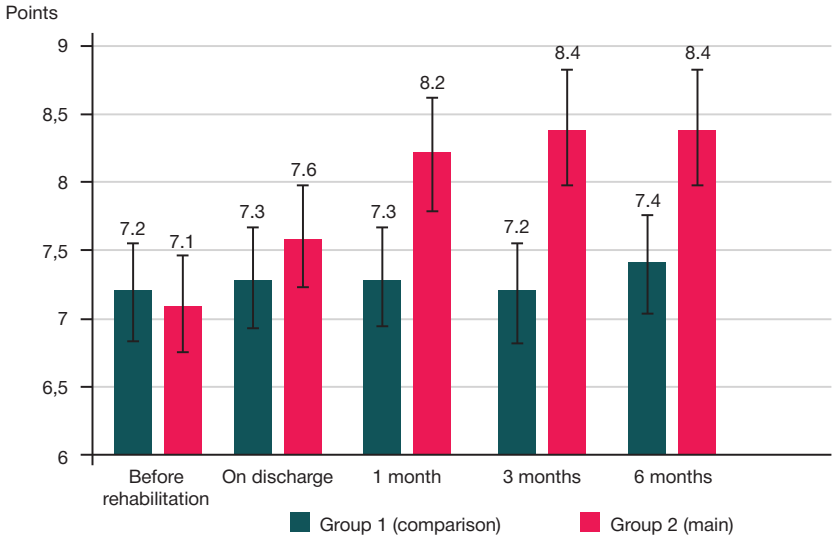

Fig. 5. Dynamics of the total MMSE score, M ± *m*

Table. Dynamics of SS-QoL scores, M ± *m*

	Before rehabilitation		At 6 months	
	Group 1 (comparison)	Group 2 (main)	Group 1 (comparison)	Group 2 (main)
Energy	2.72 ± 0.22	2.63 ± 0.24	2.60 ± 0.17	3.07 ± 0.15*
Family roles	2.66 ± 0.32	2.62 ± 0.18	2.43 ± 0.21	3.72 ± 0.32*
Language	3.91 ± 0.19	3.85 ± 0.26	3.81 ± 0.16	3.74 ± 0.26
Mobility	3.30 ± 0.35	3.56 ± 0.23	2.92 ± 0.37	4.31 ± 0.29*
Mood	3.21 ± 0.21	3.42 ± 0.40	2.85 ± 0.28	4.34 ± 0.41*
Personality	3.24 ± 0.17	3.31 ± 0.31	3.13 ± 0.32	3.32 ± 0.22
Self-care	4.07 ± 0.42	4.24 ± 0.25	3.82 ± 0.20	4.70 ± 0.19*
Social roles	3.09 ± 0.33	2.80 ± 0.17	2.74 ± 0.17	3.82 ± 0.25*
Thinking	2.62 ± 0.27	2.63 ± 0.15	2.51 ± 0.24	2.61 ± 0.16
Upper extremity function	4.35 ± 0.21	4.52 ± 0.14	4.03 ± 0.19	4.74 ± 0.21*
Vision	2.93 ± 0.12	3.05 ± 0.22	2.72 ± 0.21	2.92 ± 0.15
Work, productivity	2.51 ± 0.17	2.62 ± 0.26	2.41 ± 0.15	3.20 ± 0.28*

Note: * — differences are significant ($p < 0.05$) relative to the comparison group (the Mann–Whitney *U* test).

survivors [25, 26]. In spite of almost zero gains in functional independence, participation of stroke survivors in social activities increases their subjective satisfaction and quality of life [27, 28].

Effective post-stroke rehabilitation requires a multifaceted multidisciplinary approach. According to the literature, rehabilitation is more effective when the patient's experience and feedback are taken into account. Active participation of the patient is important for establishing positive relationship between the patient and the therapist and for ensuring that there is no breach of ethics, especially in patient-oriented rehabilitation programs [26, 28].

In Russia, many rehabilitation facilities for neurology patients provide social rehabilitation services. They offer complex rehabilitation programs for the disabled that include social integration; this improves social adaptation, allows them to take vocational training and find a new job [14, 21].

CONCLUSIONS

The standardized program for medical and social rehabilitation of stroke survivors with moderate impairments was effective in terms of its clinical, psychological and social outcomes. The scales and inventories used in the study (specifically the Rivermead Mobility Index, DASH and the SS-QoL questionnaire) are sensitive to changes in the functional state of stroke patients with moderate impairments. More initiatives are needed to raise awareness and provide consultations on post-stroke adaptation, organize social, cultural, sports and educational events. It is important to conduct studies that provide the rationale for and refine the systemic approach to planning and implementing social support services for stroke survivors. Using such approaches in rehabilitation practice will increase the level of social support for stroke survivors, help them to recover the affected functions, encourage patients to actively participate in social activities and thus improve patient outcomes.

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PREVALENCE OF RISK FACTORS FOR HEALTH AND EMOTIONAL WELL-BEING OF TEACHERS IN THE CONTEXT OF DISTANCE LEARNING

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Distance learning (DL) changed the work-rest balance of teachers, increased risks of deterioration of their health and emotional burnout (EB). This study aimed to investigate the prevalence of risk factors affecting health and emotional state of teachers engaged in DL, as well as subjective assessment of the significance of these factors by teachers. We have surveyed teachers during traditional, in-person learning (TL) period (n = 224) and DL (n = 619), and took anthropometric measurements of 45 teachers during TL and 72 teachers when DL ended. EB was studied in 72 teachers with the help of V.V. Boyko questionnaire. Statistical processing was enabled by the Statistica 13 PL package, Student's t-test, χ^2 test; to identify the relationship between indicators, we relied on regression analysis, effect occurrence probability calculation. Less than half of teachers are aware of the health risk factors (low level of physical activity — 36.1%, poor nutrition — 29.2%, lack of knowledge on disease prevention — 6.9%). After DL ended, only 30% of teachers considered themselves healthy; 13.1% reported lack of EB symptoms. An increase in the average body mass index value was established. Teachers underestimating EB and health risk factors were 2.3 times more likely to grow obese (OR = 0.40; 95% CI = 0.22–0.70). The study highlights high hygienic value of physical activity as a controllable health risk factor. Raising teachers' awareness of health preservation practices will help prevent deterioration of their health, development of EB and increase the efficiency of their professional activity.

Keywords: teacher, distance learning, health risk factor, emotional state, emotional burnout

Author contribution: Milushkina OYu — study management; Zhukov OF, Markelova SV, Lukanova OV — material collection, statistical processing, article authoring; Skoblina NA — literature analysis.

Compliance with ethical standards: the study was approved by the Ethics Committee of Pirogov Russian National Research Medical University (Protocol № 159 of November 21, 2016). Each participant signed a voluntary informed consent form. Adult population participated voluntarily, through filling in questionnaires online.

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РАСПРОСТРАНЕННОСТЬ ФАКТОРОВ РИСКА НАРУШЕНИЯ ЗДОРОВЬЯ И ЭМОЦИОНАЛЬНОГО БЛАГОПОЛУЧИЯ УЧИТЕЛЕЙ В УСЛОВИЯХ ДИСТАНЦИОННОГО ОБУЧЕНИЯ

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Дистанционное обучение (ДО) изменило режим труда и отдыха учителей, увеличило риски нарушения состояния их здоровья и наступления эмоционального выгорания (ЭВ). Целью исследования было изучить распространенность факторов риска, воздействующих на здоровье и эмоциональное состояние учителей в условиях проведения ДО, и субъективную оценку учителями значимости этих факторов. Проведено анкетирование учителей в период традиционного обучения (ТО) (n = 224) и ДО (n = 619), антропометрическое обследование 45 учителей в период ТО и 72 учителей по завершению ДО. Изучено ЭВ у 72 учителей с использованием опросника В. В. Бойко. Для статистической обработки использовали пакет Statistica 13 PL, t-критерий Стьюдента, критерий χ^2; для выявления взаимосвязи показателей — регрессионный анализ, расчет шансов возникновения эффекта. Менее половины педагогов информированы о факторах риска нарушения здоровья (низкая двигательная активность — 36,1%, нерациональное питание — 29,2%, отсутствие знаний по профилактике заболеваний — 6,9%). После завершения ДО только 30% учителей считали себя здоровыми, 13,1% отметили отсутствие симптомов ЭВ. Установлено увеличение среднего значения индекса массы тела. Шансы развития избытка массы тела и ожирения у учителей, недооценивающих факторы риска развития нарушений здоровья и ЭВ, в 2,3 раза выше (ОШ = 0,40; 95% ДИ = 0,22–0,70). Показано высокое гигиеническое значение двигательной активности как управляемого фактора риска нарушения состояния здоровья. Повышение информированности учителей в вопросах здоровьесбережения позволит сохранить их здоровье, предупредить развитие ЭВ и повысить эффективность профессиональной деятельности.

Ключевые слова: учитель, дистанционное обучение, фактор риска здоровью, эмоциональное состояние, эмоциональное выгорание

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In 2020, with COVID-19 pandemic and quarantine measures in the background, over 1.5 billion children, adolescents and youth were switched to distance learning (DL). Distance learning implies interaction of teachers and students at a distance, with all the components of educational process (goals, content, methods, organizational patterns, teaching aids) factored in. Distance learning relies on the specific constituents of the internet technology [1, 2].

Schools were forced to switch to distance learning strategies, but their experience of application thereof was limited [3].

Studies by both Russian and foreign researchers and educators have shown that switch to the distance mode of learning entailed increased loads on all educational process participants (teachers, students, parents) [4–9], which caused disorders in general and distressed emotional well-being of teachers in particular [10]. According to the studies, 34% of teachers experienced anxiety during a pandemic and 8% exhibited strong depressive emotions. It was also established that women show positive correlation with fear and depression and negative correlation with optimism [10, 11]. The vast majority of education professionals are women [12].

Teachers who relied on information and communication technologies in their work during the COVID-19 pandemic noted that this form of work was a source of significant stress for them, which, as factored in the teacher success model based on the theory of self-determination, can cause emotional burnout [13].

The professional activity of a teacher involves close communication with students in an emotionally rich atmosphere. Emotional burnout is one of the possible fallouts thereof. Emotional burnout (EB) is a dynamic process that develops in stages, three of them. At the first stage, nervous tension rises from the atmosphere of chronic psychoemotional instability, destabilizing environment, increased responsibility, difficulties inherent to interactions with the given group of pupils/students. At the second stage, the person undergoing emotional burnout shows resistance, i.e., tries to develop protection against unpleasant impressions, with varying results. At the third stage, this person suffers depletion of mental resources, substandard emotional tone, both being the result of failure to resist at the second stage.

For teachers, DL means altered work and rest regimes, increased number of electronic devices in use, greater screen time associated with work (value several times as great as it was before DL), increased loads on the visual analyzer, musculoskeletal system. The way of life of teachers has changed, they were subjected to a significantly more intense neuro-emotional stress, and the loads on individual functional systems of the body have increased [14]. The peculiarities of organization of educational process in the DL mode, with forced self-isolation in the background, constitute an additional emotional burnout risk factor for teachers. This risk factor was only addressed in a small number of studies [15].

Thus, the problem of preserving health of teachers in the context of educational process intensification, growing reliance on information and communication technologies as part of the digital educational environment deployment, is of great scientific interest.

This study aimed to investigate the prevalence of risk factors affecting health and emotional state of teachers engaged in DL, as well as subjective, teachers' assessment of the significance of these factors for health.

METHODS

We developed special online questionnaires to collect teachers' subjective assessments of their health, capability to follow a healthy lifestyle (HLS) when teaching traditionally (traditional learning, TL) and in the distance learning (DL) mode. The questionnaires were published as and distributed through Google Forms [16].

In 2019, we had 224 teachers fill in the online questionnaires during the TL period. In April 2020, during the DL period, we collected filled questionnaires from 619 teachers. The respondents lived in Moscow and the Moscow region, Yekaterinburg, Nizhny Novgorod, Voronezh, Ulyanovsk and other cities. Most of them were women. The average age of the respondents was 47.1 years. The questionnaires included questions aimed at collecting information about respondents' subjective assessment of their health; level of commitment to regular exercising (sports and other types of physical activity), eating at least three times a day, monitoring energy value of food; awareness about risk factors that can have a negative impact on health and emotional well-being.

Anthropometric measurements (body length and weight) were taken in 2019 during the TL period (45 teachers) and in 2021, when the DL period ended (72 teachers). Body mass index assessment followed the WHO standard, which considers values from 18.5 to 24.9 normal.

In February 2021, when the DL period has ended, we surveyed 72 teachers online, asking questions to collect data on their health status, anthropometric indicators, the influence of various factors on health and emotional well-being [17]. We also used V.V. Boyko questionnaire to identify EB in teachers and to establish groups of external and internal factors triggering it [18]. The group of organizational (external) factors included work conditions and socio-psychological activity (chronic psycho-emotional stress, unclear work organization and planning patterns, increased responsibility for the results of functional execution, unfavorable psychological atmosphere inherent to the professional activity). The group of internal factors included inclination to be emotionally rigid, intense internalization (perception and experience) of the circumstances of professional activity, weak motivation for emotional return in professional activity [18].

Factoring in the identified health risk factors peculiar to work and life of teachers, we built a regression model that describes their subjective assessment of their health in points (subjective positive assessment of health at $Y \leq 1$).

We calculated the odds ratio of developing overweight and obesity among teachers who adequately assessed and underestimated the health and emotional well-being risk factors.

Statistical data processing relied on Statistica 13.0 (StatSoft Inc.; USA), Student's t-test, χ^2 test; the differences were considered significant at $p \leq 0.05$.

RESULTS

Only a third of teachers (30%) of general primary and secondary education establishments considered themselves healthy.

During the TL period, every fifth teacher (21.4%) underestimated the health risk associated with lack of regular exercise sessions (sports and other types of physical activity), every third (28.6%) teacher underestimated the risk associated with having less than three meals a day, every second (55.8%) teacher paid no attention to the energy value of food consumed.

DL had a specific effect on the way of life of teachers. The majority of respondents (94.1%) reported decreased physical activity level; only half of them (56.2%) mentioned maintaining physical activity level at the minimum. During the TL period, every second teacher (45.5%) monitored his/her level of

physical activity, while during the DL period it was only every third teacher (33.0%) ($p \leq 0,05$).

Teachers examined when the DL period ended had their the average body mass index (BMI) at 26.9 ± 0.7, while during the TL period it was at 25.7 ± 0.7; every fourth (23.8%) had some degree of obesity, every third (38.1%) was overweight; about a third (38.9%) of the respondents mentioned getting sick more often during the DL period, and only every seventh (15.3%) teacher reported no sickness incidents at all.

After the DL period, only every eighth teacher (13.1%) reported lack of EB symptoms. The remaining respondents mentioned presence of certain emerging or established EB symptoms.

At the "tension" stage, every second teacher (45.9%) had a developed "experiencing traumatic circumstances" symptom, while every third (31.2%) exhibited the "anxiety and depression" symptom.

At the "resistance" stage, half of the respondents signaled high values of the "inadequate selective emotional response" (54.1%) and "professional deterioration" (50.8%) symptoms; a third of them noted "emotional and moral disorientation" (32.8%), "expanded emotional numbness field" (31.1%).

At the "depletion" stage, every fourth teacher (22.9%) had the "psychosomatic and psychovegetative disorders" symptom manifesting or dominating. After the DL period was over, teachers listed the risk factors that, in their opinion, had an impact on health and emotional state (Fig.).

According to the teachers surveyed, the risk factors affecting health and possible EB onset during the DL period were: work intensity, checked by every second respondent (61.1%); low physical activity, checked by every third respondent (36.1%); inappropriate nutrition (29.2%); lack of timely medical care (29.2%). Every fifteenth respondent mentioned "lack of knowledge on disease prevention" as a risk factor (6.9%).

Teachers underestimating emotional well-being and health risk factors were 2.3 times more likely to grow obese (OR = 0.40; 95% CI = 0.22–0.70). We built a regression model that incorporates the most informative variables describing health status and EB development in teachers: age and gender characteristics, level of physical activity ($p \leq 0.05$):

$$Y = 1.48 + 0.23X1 - 0.80X2,$$

where X1 — age and gender characteristics; X2 — the level of physical activity.

The results highlight high hygienic value of physical activity as a controllable health risk factor.

DISCUSSION

The vast majority of general education teachers turned out to have no DL experience both in Russia and all over the world.

During the COVID-19 pandemic, school teachers did gain some experience against the background of need to adapt to the unprecedented DL process [18–20].

Analyzing the results of distance learning, pedagogical community realized the need to investigate physical and mental health status of all participants of the educational process occurring without in-person contact. Psychologists and teachers, physicians and physiologists state that in the absence of immediate live interaction of students and teachers it is more difficult for the former to perceive, understand and memorize the material presented, with the neuropsychic load suffered by the participants of such educational process increasing severalfold, which calls for adaptation of educational material, teaching methods and forms to fit the DL requirements [21–29].

The announced self-isolation regime, the switch to DL and the associated need to master information and communication technologies within a limited period of time changed lifestyle of teachers, increased their work hours and the intensity of work, which can be regarded as an additional risk factor for the teachers' health and emotional sphere.

This testifies to the lack of teachers' awareness of healthy lifestyle practices, non-adherence thereto in daily and professional activities, which, with the growing deficit of physical activity and work intensity, contributed to physical health disorders, obesity and excess body weight and disturbed emotional well-being. The study has shown the importance of physical activity as a manageable risk factor in the context of health disorders and development of emotional burnout in teachers.

CONCLUSIONS

This study allowed establishing the fact that teachers underestimate the health and emotional well-being risk factors. The situation was obvious during the TL period and deteriorated further during the DL period. We have shown the high value of

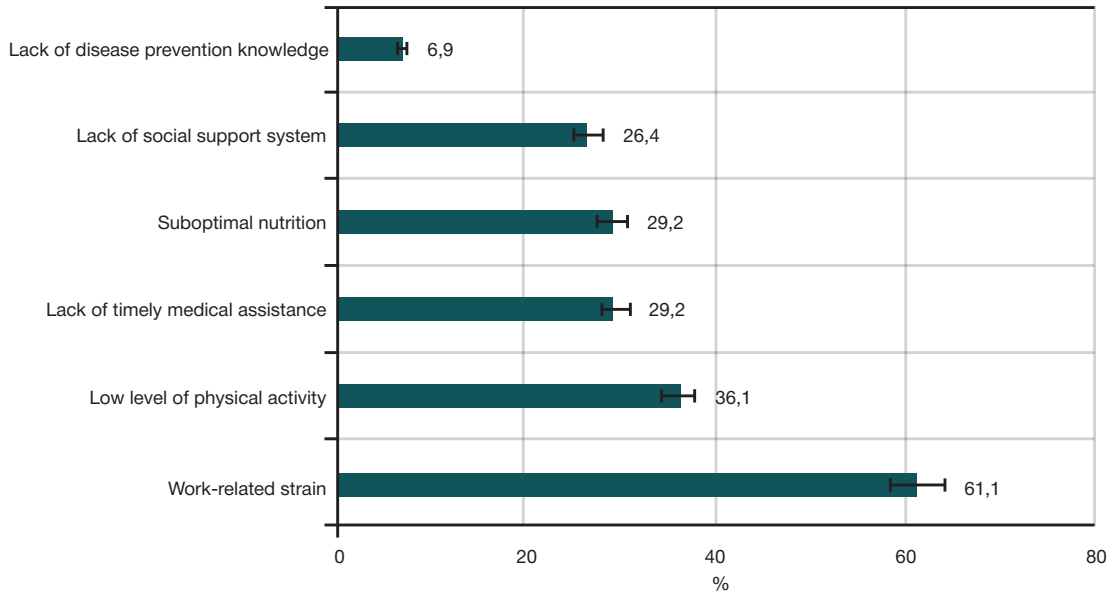

Fig. Risk factors that, according to the teachers, have affected their health and emotional state during the DL period

an adequate subjective assessment of health and emotional well-being risk factors associated with physical activity, rational nutrition, the possibility of receiving timely medical care as components of a healthy lifestyle, as well as the high hygienic value of the level of awareness of healthy lifestyle practices health disorder prevention. The study underscores the high relevance of involving teachers in hygienic education and healthy lifestyles promotion activities. Such efforts will have a positive effect on their health and emotional well-being, including in the context of introduction of information and communication technologies into their professional lives, and will also increase the level of subjective assessment of the health risk factors.

The experience gained from long-term distance work exacerbated the existing problems resulting from the intensive introduction of information and communication technologies into the educational process, helped identify health and emotional well-being risk factors affecting all participants of the educational process, allows studying teachers' subjective assessment of existing risk factors, outlining priority areas for prevention efforts in the context of giving hygienic education to the teachers. Deployment of preventive programs for teachers, including those covering safe use of communication devices, work and rest balance, rational nutrition principles, optimal physical activity will not only help prevent deterioration of their health, preserve emotional well-being, but also increase their professional effectiveness and, consequently, the level of education received by their students.

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