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POSSIBILITIES OF APPLYING PHYSIOTHERAPEUTIC TREATMENT METHODS FOR THE PURPOSE OF PREVENTION OF RESTRICTIONAL DISORDERS OF EXTERNAL RESPIRATORY IN PATIENTS AFTER BREAST ENDOPROSTHETICS

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Abstract. ***Introduction.*** Implantation of foreign silicone implants during breast replacement is accompanied by structural and functional changes in the surrounding tissues. Developing chronic inflammation, fibrosis, severe pain and the compression effect of the implant lead to severe restrictive disorders of external respiration functions, reducing the patient's quality of life in the postoperative period. One of the possible ways to solve this problem is to use an electromagnetic field with a frequency of 448 kHz. ***Purpose of the study:*** to evaluate the effectiveness of physiotherapeutic methods of influence as methods for the prevention of restrictive disorders of external respiration in patients after breast replacement. ***Materials and methods.*** The study is based on the results of a survey of 89 females who underwent breast replacement with silicone implants. All women were divided into 4 groups, taking into account the approach to the use of physiotherapy. The study assessed respiratory rate, vital capacity, forced vital capacity, formed expiratory volume in the first second, and peak expiratory volumetric flow rate. ***Results.*** It was found that all patients in the early postoperative period had impairment of external respiratory function by an average of 30% of the reference values. The combined use of electrophysiological effects and botulinum toxin made it possible to reduce the frequency of identified respiratory system disorders. By the 7th day of treatment, the frequency of shortness of breath, as well as the magnitude of the deviation from the norm of vital capacity of the lungs, forced vital capacity of the lungs and forced expiratory volume were less, respectively, by 12.3 times, 6.4 times, 8.7 times. 8.1 times compared to control. ***Conclusion.*** A set of preventive measures, including electrophysiological therapy, can significantly increase the efficiency of restoration of external respiratory functions in patients after breast replacement in the first week after surgery.

Key words: breast replacement; external respiration disorders; spirometry; botulinum toxin; electrophysiological effects.

ВОЗМОЖНОСТИ ПРИМЕНЕНИЯ ФИЗИОТЕРАПЕВТИЧЕСКИХ МЕТОДИК ЛЕЧЕНИЯ С ЦЕЛЬЮ ПРОФИЛАКТИКИ РЕСТРИКТИВНЫХ НАРУШЕНИЙ ВНЕШНЕГО ДЫХАНИЯ У ПАЦИЕНТОК ПОСЛЕ ЭНДОПРОТЕЗИРОВАНИЯ МОЛОЧНЫХ ЖЕЛЕЗ

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Резюме. ***Введение.*** Имплантация инородных силиконовых имплантов во время эндопротезирования молочных желез сопровождается структурными и функциональными изменениями окружающих тканей. Развивающееся хроническое воспаление, фиброз, выраженный болевой синдром и компрессионное воздействие импланта приводит к выраженным рестриктивным нарушениям функций внешнего дыхания, снижающим качество жизни пациентки в послеоперационном периоде. Одним из возможных путей решения данной проблемы является применение электромагнитного поля с частотой 448 кГЦ. ***Цель исследования*** — оценить эффективность физиотерапевтических методик воздействия в качестве методов профилактики рестриктивных нарушений внешнего дыхания у пациенток после эндопротезирования молочных желез. ***Материалы и методы.*** Исследование основано на результатах обследования 89 лиц женского пола, перенесших эндопротезирование молочных желез силиконовыми имплантами. Все женщины были разделены на 4 группы с учетом подхода к использованию физиотерапевтического метода воздействия. В ходе исследования оценивалась частота дыхания, жизненная емкость легких, форсированная жизненная емкость легких, объем форсированного выдоха за первую секунду, пиковая объемная скорость выдоха. ***Результаты.*** Установлено, что у всех пациенток в раннем послеоперационном периоде отмечалось нарушение функций внешнего дыхания в среднем на 30% от референсных значений. Комбинированное использование электрофизиологического воздействия и ботулотоксина позволило снизить частоту выявленных нарушений дыхательной системы. К седьмым суткам лечения частота одышки, а также величина отклонения от нормы жизненной емкости легких, форсированной жизненной емкости легких и объема форсированного выдоха оказались меньше, соответственно, в 12,3 раза, в 6,4 раза, в 8,7 раза и в 8,1 раза по сравнению с контролем. ***Заключение.*** Комплекс профилактических мероприятий, включающий электрофизиологическую терапию, позволяет достоверно повысить эффективность восстановления функций внешнего дыхания у пациенток после эндопротезирования молочных желез в первую неделю после операции.

Ключевые слова: эндопротезирование молочных желез; нарушения внешнего дыхания; спирометрия; ботулотоксин; электрофизиологическое воздействие.

INTRODUCTION

At present, breast replacement is one of the most demanded surgical interventions in aesthetic medicine [6]. Annually, no less than 100.000 surgeries with silicone implant use are performed in plastic surgery clinics not only in Russia but abroad as well, and these data do not tend to decline but steadily grow [4].

Breast enlargement and change of its structure is gained by the use of implant mostly on the hydro-gel silicone basis [8]. Installation of the latter in the chest results in a number of possible physiological and functional changes often leading to pathological conditions, e.g. pneumonitis. V.S. Paredes et.al have demonstrated by their study (2010) that approximately in one third of all women who underwent breast replacement with silicone implants infiltrates in the parenchymatous tissue of the lung were identified within the 1st week after the surgery [10]. At the same time, there was a chronic inflammatory process in the tissues surrounding the foreign implant,

which was a normal body response. This provides silicone fibrous encapsulation and pulmonary tissue fibrosis with participation of macrophages, T-cells as well as active B-lymphocytes [1, 7].

Pain syndrome, an expected surgical intervention effect, is one of the most significant problems in mammary gland endoprosthetics. Trauma of anatomical structures, as well as excessive overstretching of tissues cause constant pain impulsation development which may persist for several years following the surgery [11]. This results in emotional health deterioration and lowed satisfaction from breast implantation.

Abnormal changes of the soft tissues surrounding silicone implant as well as of the lung parenchyma combined with surgical interventions in the region of the thorax lead to marked restrictive impairment of external respiration functions [9]. Moreover, compressing effect of a foreign body in the region of the chest initiates these changes leading to new chains of pathogenesis developing with the latter joining to vicious circles. Numerous findings of spirographic studies of females after breast implantation show significant reduction in all available external respiration data [3]. Absence of possibility to ventilate the lungs properly lowers quality of life in both the early and the late postoperative period [2].

One of the possible ways of accelerated arresting of tissue inflammatory changes and pain syndrome following the mammary gland endoprosthetics is the use of an electromagnetic field with a frequency of 448 kHz that activates ion exchange due to which natural cell regeneration processes are effected more efficiently. Such physiotherapeutic equipment makes electric potential of the cell membrane restored in the postoperative period, activates collagen production; improves microcirculation and tissue trophicity, produces antiedematous effect, promotes hematoma reorganization and stem cells proliferation as well [5]. Noted properties are of particular interest considering their use to prevent restrictive impairment of external respiration in patients after breast endoprosthetics.

OBJECTIVE OF THE STUDY

To evaluate the effectiveness of physiotherapeutic methods of influence as methods for the prevention of restrictive disorders of external respiration in female patients following breast replacement.

MATERIALS AND METHODS

An open, randomized, mono-centered and prospective study has been carried out to evaluate the possibility of the use of physiotherapeutic methods as preventive measures of external respiration restrictive impairment after mammary gland endoprosthetics. The work is based on the findings of 89 implantations with the silicone implants use performed in the Plastic Surgery Unit of the “Relax Med Service” clinic in Samarkand, the republic of Uzbekistan for the period of 2021–2023.

Female patients were involved into the study according to the following criteria: aged 25–50, the presence of clinically meaningful hypomastia, asymmetry of the mammary glands, no earlier surgeries in the regions of the chest and breast, patient’s voluntary consent to be involved in the study on the assessment of postoperative rehabilitation measures effectiveness.

Female patients were not involved under the following conditions: the age under 25 and over 50 years, some chronic diseases as well as their acute stage, CAD, obstructive lung disease, respiratory failure of any degree, skin infectious and non-infectious diseases in the region of the chest, hyper and hypocoagulation, HIV, previous hepatitis B, C, tuberculosis, any gestational age, lactation, cardiac pacemaker, thrombophlebitis, refusal to be involved in the study on the assessment of postoperative rehabilitation measures effectiveness.

All the patients were divided into four groups considering pre- and postoperative management: botulinum toxin type A injection into the *musculus pectoralis major* 14 days before endoprosthetics (n=23), botulinum toxin type A injectioon into the *musculus pectoralis major* 14 days before endoprosthetics with the postoperative 1 week physiotherapeutic treatment with the help of the INDIBA apparatus (n=24), 0.9% saline solution injection into the *musculus pectoralis major* 14 days before endoprosthetics with the postoperative 1 week physiotherapeutic treatment with the INDIBA apparatus (n=22), 0.9% saline solution injection into the *musculus pectoralis major* 14 days before endoprosthetics (control, n=20).

The botulinum toxin type A “Botox” in the dose of 100 units was injected bilaterally in concentration 1 to 25 in 10 symbolic muscular sectors presented in Figure 1. The amount of the preparation administered into one spot did not exceed 2.5 ml, 0.9% saline solution was injected similarly.

Electrophysiological therapy of the chest region was conducted by means of the INDIBA active 801 apparatus (Spain). The procedure of radiofrequency cellular electrotherapy was performed with a frequency of 448 kHz in capacity regime within 15 minutes daily during the 1st weak after the surgery.

The assessment of external respiration was carried out by determining spirometry findings a day before breast endoprosthetics and on the 1st and the 7th day following the

operation. Instrumental investigation was conducted with the help of microprocessor portable spirograph SMP-21/01-R-D (Scientific and Production Enterprise "Monitor", Russia). Registration and analysis of dynamic findings of the vital capacity of the lung (VCL), forced vital lung capacity (FVLC), forced expiratory volume in the 1st second (FEV_1), peak expiratory flow rate (PEFR) were performed according to recommendations of the Russian Respiratory Society.

Statistic processing of the study data used common methods of variation statistics. To assess the significance of differences student's t-test was used. The belonging of the samples to the normal distribution was determined using the Kolmogorov–Smirnov criterion. An alternative hypothesis was accepted at $p < 0.05$.

STUDY RESULTS AND THEIR DISCUSSION

While analyzing parameters of external respiration and spirograms of 89 females it was identified that mammary gland endoprosthetics with silicone implants caused tachypnea developing in 59.5% of observed cases in the first day after the trauma. By the second day, the analyzed parameter reduced in up to 40.4% of cases (32 patients) and reached its minimum by the end of the week in 9 patients or 10.1% of observed cases.

Analysis of VCL, FVLC, FEV_1 and PEFR parameters in all patients on the 1st day after breast implantation showed their significant reduction, correspondently by 26.8, 31.1, 29.2 and 25.3% ($p < 0.05$). During the next week, follow-up disorders became less prominent. Assessed parameters reduced only by 8.9, 11.2, 7.4, and 13.2% ($p > 0.05$) (Table 1).

In-depth analysis of the presented in Table 1 data makes it possible to conclude that decrease of respiratory volume and external respiration parameters taken within the first 24 hours after breast replacement is due to restrictive disorders characterized by severe pain syndrome and limited chest excursion because of the silicone implant.

The findings of assessment of external respiration and spirogram parameters in four patients' groups divided according to the way of arresting pathological conditions progressing following breast endoprosthetics are given in Table 2.

Examination findings presented in Table 2 testify that within first 24 hours after the surgery on aesthetic mammary gland endoprosthetics in the group of patients who were preliminary administered botulinum toxin type A dyspnea was recorded in 31.2% of cases. At the same time VLC, FVLC, FEV_1 and PEFR reduced by minimum 30% compared to their values before the surgery in 34.1, 22.8, 33.2 and 12.1% of observed cases. By the 7th day of follow up the noted parameters were found to be restored compared to the first day of postoperative period by 150–200% ($p < 0.05$).

When a combination of botulinum toxin type A with a course of physiotherapeutic treatment INDIBA is

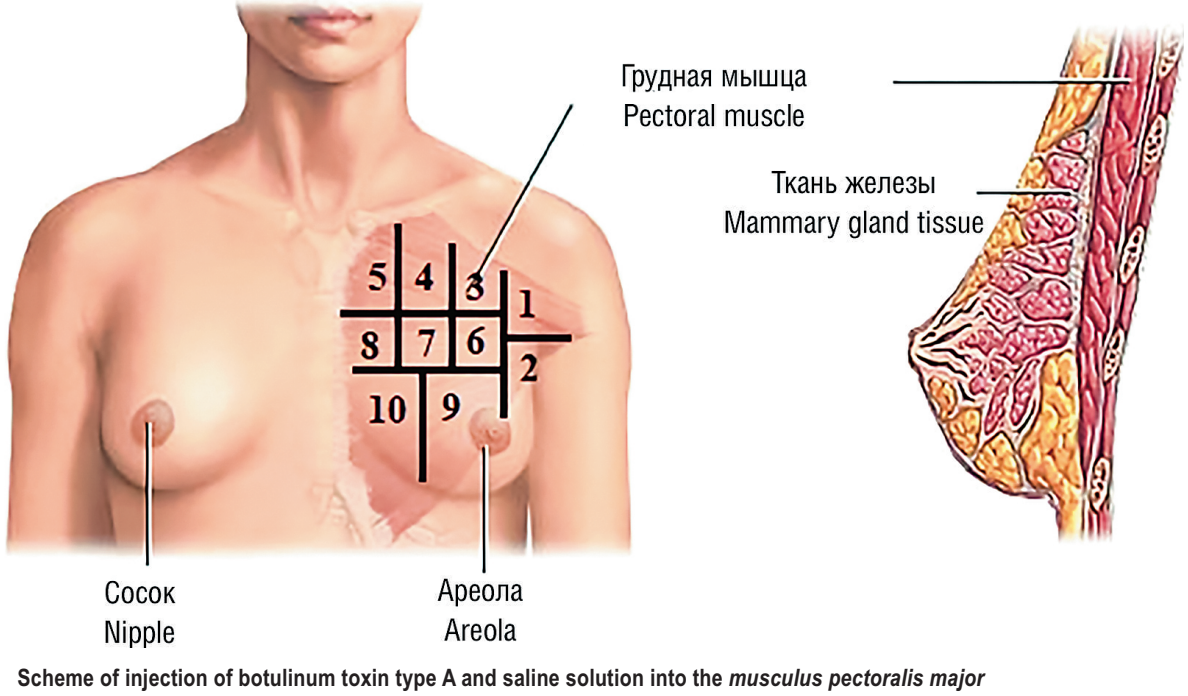

Fig. 1. Scheme of injection of botulinum toxin type A and saline solution into the *musculus pectoralis major*
Рис. 1. Схема инъекционного введения ботулотоксина типа А и 0,9% натрия хлорида в *musculus pectoralis major*

Table 1

Spirometry parameters in the first week after breast replacement

Таблица 1

Параметры спирометрии в первую неделю после эндопротезирования молочных желез

Анализируемые параметры / Studied parameters	Величина анализируемых параметров в группе / The value of the analyzed parameters in the group			
	срок, сут / duration, days	среднее, M±m / average, M±m	число наблюдений (%) / number of observations (%)	
			в норме / normal	вне нормы / not normal
Частота дыхания, л / Respiration rate, l	1	23,3±2,4	36 (40,5)	53 (59,5)
	7	18,4±2,5	80 (89,9)	9 (10,1)
Жизненная емкость легких, л / Vital capacity of the lungs, l	1	74,2±6,2	37 (41,6)	52 (58,4)
	7	83,1±7,8	64 (72,0)	25 (28,0)
Форсированная жизненная емкость легких, л / Forced life lung capacity, l	1	79,4±6,6	35 (39,3)	54 (60,7)
	7	85,1±7,3	66 (74,1)	25,9 (3,5)
Объем форсированного выдоха, л / Forced volume exhalation, l	1	65,1±5,1	40 (44,9)	49 (55,1)
	7	71,4±6,3	72 (80,8)	17 (19,2)
Пиковая объемная форсированная скорость выдоха, л/с / Peak volumetric forced expiratory flow, l/s	1	76,8±8,2	70 (78,7)	19 (21,3)
	7	80,2±6,3	81 (91,1)	8 (8,9)

Table 2

Respiratory system examination results after breast replacement

Таблица 2

Результаты обследования дыхательной системы после эндопротезирования молочных желез

Срок, сут / Duration, days	Подгруппа / Subgroup	Частота выявления нарушений, % / Frequency of detection of violations, %				
		одышки / dyspnea	жизненной емкости легких / vital capacity of the lungs	форсированной жизненной емкости легких / forced vital capacity	объема форсированного выдоха / forced expiratory volume	пиковой объемной форсированной скорости выдоха / peak forced expiratory volumetric flow rate
1	1	31,2	34,1	22,8	33,2	12,1
	2	35,4	32,2	24,5	31,2	13,6
	3	43,3	47,1	46,4	37,5	24,8
	4	96,4	98,1	96,1	100	34,5
7	1	18,4	16,8	18,4	21,1	0
	2	1,5	2,6	2,1	2,6	0
	3	23,1	26,3	21,5	19,4	0
	4	54,3	43,8	41,4	37,5	0
p между 1 и 2 / *p* between 1 and 2	1-е сут	>0,05	>0,05	>0,05	>0,05	>0,05
	7-е сут	<0,01	<0,05	<0,01	<0,05	0
p между 1 и 4 / *p* between 1 and 2	1-е сут	<0,01	<0,01	<0,01	<0,01	>0,05
	7-е сут	<0,05	<0,05	<0,05	>0,05	0

used the prevalence of identified expiratory respiration function disorders differed from the value of the corresponding parameters in the first group not more than by 8–14% (p>0.05). By the end of the week the prevalence of dyspnea, VLC, FVLC, FEV_1 turned out to be less by 12.3 times (p <0.05), 6.4 times (p <0.05), 8.7 times (p <0.05), 8.1 times (p <0.05) correspondingly compared to the findings of the group where patients received only botulinum toxin type A.

In the control group at both points of examination in most clinical cases, impaired external respiration functions were marked. On the first day of the postoperative period the frequency of dyspnea as well as parameters of VLC, FVLC, FEV_1 and PEFR were minimum 30% below the normal level correspondingly in 96.4, 98.1, 96.1, 100 and 34.5% of cases. On the seventh day of the examination, the frequency of mentioned impairments decreased almost by half, but had significant statistical differences with the first and second study groups data where botulinum toxin type A was combined with physiotherapeutic treatment INDIBA.

External respiration and spirogram parameters findings in the third group that received isolated physiotherapeutic therapy with INDIBA apparatus were analyzed separately (Table 3).

Spirograms results given in Table 3 show that within the whole course of physiotherapy the respiration frequency tended to decrease till 19 per minute compared to 22 per minute recorded in the group where patients received placebo (p >0.05). After seven days of electrophysiological exposure VLC, FEV_1 and PEFR parameters turned out to be higher than control only by 5, 4 and 5% respectively (p >0.05). Considering data received on the basis of a profound calculation with the use of student's t-test none statistically significant differences in spirogram parameter values in the postoperative period after endoprosthetics of the breast taking into consideration electrophysiological effect were revealed. One may conclude about the absence of a proven effect of the isolated use of INDIBA apparatus on external respiration function in the postoperative period in our follow-up groups.

CONCLUSIONS

1. Breast endoprosthetics is accompanied by marked restrictive disorders of patients' external respiratory functions such as reduction of vital lung capacity, forced vital lung capacity, forced expiratory volume in the 1st second and peak expiratory flow rate. The above mentioned spirometry parameters changes reached their peak in the first week following the aesthetic surgery.

2. Isolated physiotherapeutic treatment with INDIBA apparatus did not produce any significant effect on the rate of expiratory respiration functions restoration in female patients after mammary gland replacement.

3. A set of preventive measures based on the botulinum toxin type A administration in the large pectoral muscle before the operation as well as a course of anti-inflammatory electrophysiological therapy make it reliably possible

Table 3

Respiratory system examination results after a week after endoprosthetics of mammary glands, taking into account electrophysiological effects

Таблица 3

Результаты обследования дыхательной системы через неделю после эндопротезирования молочных желез с учетом электрофизиологического воздействия

Анализируемые параметры / Analyzed parameters	Средняя величина параметров при воздействии INDIBA / Average parameter values when exposed to INDIBA		Разность средних / Difference average	*p*
	есть yes	нет no		
Частота дыхания, л / Respiration rate, l	$_{18}$ 19 $_{21}$	$_{20}$ 22 $_{23}$	$_{-0,5}$ –1,0 $_{0,5}$	0,05
Жизненная емкость легких, л / Vital capacity of the lungs, l	$_{79}$ 81 $_{82}$	$_{76}$ 77 $_{79}$	$_{-0,7}$ –0,6 $_{0,8}$	0,61
Объем форсированного выдоха, л / Forced volume exhalation, l	$_{77}$ 78 $_{79}$	$_{74}$ 75 $_{77}$	$_{-0,1}$ –0,2 $_{0,6}$	0,06
Пиковая объемная форсированная скорость выдоха, л/с / Peak volumetric forced expiratory flow, l/sec	$_{82}$ 83 $_{85}$	$_{77}$ 79 $_{80}$	$_{-0,3}$ –0,2 $_{0,7}$	0,07

Примечание: границы 95% доверительных интервалов для медианы подстрочно.
Note: limits of 95% confidence intervals for the median line by line.

to increase the rate of expiratory respiration functions restoration in patients following breast endoprosthetics in the first week after the surgery.

4. Combination of botulinum toxin and one of the physiotherapeutic methods provided both the complete *musculus pectoralis major* denervation and effective anti-inflammatory and metabolic effect on the injured by the surgery tissues, which resulted in marked analgesic effect development. In the absence of pain, no impaired external respiration parameters resulted from, with respiratory volume being normal and breast excursions being painless in the early postoperative period. Combined use of physiotherapy and botulinum toxin proved to be more effective than their isolated application.

ADDITIONAL INFORMATION

Author contribution. Thereby, all authors made a substantial contribution to the conception of the study, acquisition, analysis, interpretation of data for the work, drafting and revising the article, final approval of the version to be published and agree to be accountable for all aspects of the study.

Competing interests. The authors declare that they have no competing interests.

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OPTIMIZATION OF VENTRICULAR ARRHYTHMIAS DIAGNOSTICS: ASSESSING THE DYNAMICS OF THE REGULATORY-ADAPTIVE STATUS

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Abstract. ***Introduction.*** Criteria for the effectiveness of therapy for ventricular arrhythmias (VA) take into account only the antiarrhythmic effect of pharmacological agents, which does not correspond to the modern personalized approach in medicine. Currently beta-blockers (BB) have the greatest evidence base in the treatment of VA. Their hypotensive and antianginal properties have been sufficiently studied. However, there is evidence of a possible negative impact of BB on the regulatory-adaptive status (RAS), characterizing the global functional state, ability to regulate and adapt. Thus, the issues of optimizing diagnostics in patients with VA are relevant. ***Aim.*** To study the effect of β-blockers on the parameters of the cardiorespiratory synchronism (CRS) test in patients with VA. ***Materials and methods.*** The study included 120 patients with VA and essential hypertension (EH) or its combination with coronary heart disease (CHD), randomized into three groups for the treatment of BB with different pharmacochemical properties: bisoprolol, nebivolol and sotalol. As part of combination therapy, an angiotensin-converting enzyme inhibitor, lisinopril, was prescribed, and if indicated, a disaggregant, acetylsalicylic acid, and a lipid-lowering drug, atorvastatin. Initially and after 24 weeks of therapy, the following were performed: CRS test, daily monitoring of the electrocardiogram and blood pressure. ***Results.*** In all three groups of patients, comparable hypotensive and antiarrhythmic effects were recorded. When nebivolol was prescribed as part of combination therapy in patients with VA against the background of stage III EH or its combination with CHD, an increase in the synchronization range and RAS index was observed, in contrast to bisoprolol and sotalol. ***Conclusion.*** In patients with VA and EH or its combination with CHD, the CRS test makes it possible to determine the optimal combination therapy option that has the most positive effect on the functional state and RAS index.

Key words: regulatory-adaptive status; ventricular arrhythmias; bisoprolol; nebivolol; sotalol.

ОПТИМИЗАЦИЯ ДИАГНОСТИКИ ЖЕЛУДОЧКОВЫХ НАРУШЕНИЙ РИТМА СЕРДЦА: ОЦЕНКА ДИНАМИКИ РЕГУЛЯТОРНО-АДАПТИВНОГО СТАТУСА

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Резюме. ***Введение.*** Критерии эффективности терапии желудочковых нарушений ритма сердца (ЖНРС), как правило, учитывают лишь антиаритмическое действие фармакопрепаратов, что не соответствует представлению о персонифицированном подходе в медицине. Наибольшей доказательной базой в лечении ЖНРС в настоящее время обладают бета-адреноблокаторы (БАБ). В достаточной степени изучены гипотензивные и антиангинальные их свойства, однако имеются данные о негативном влиянии БАБ на регуляторно-адаптивный статус (РАС), характеризующий глобальное функциональное состояние организма, его способность к регуляции и адаптации. В связи с этим вопросы оптимизации диагностики у пациентов с ЖНРС представляются актуальными. ***Цель.*** Изучить влияние БАБ на параметры пробы сердечно-дыхательного синхронизма (СДС) у пациентов с ЖНРС. ***Материалы и методы.*** В исследование включено 120 пациентов с ЖНРС и гипертонической болезнью (ГБ) или ее сочетанием с ишемической болезнью сердца (ИБС), рандомизированных в три группы для лечения БАБ с различными фармакохимическими свойствами: бисопрололом, небивололом и соталолом. В составе комбинированной терапии назначались ингибитор ангиотензин-превращающего фермента — лизиноприл, а при наличии показаний дезагрегант — ацетилсалициловая кислота и гиполипидемический препарат — аторвастатин. Исходно и через 24 недели терапии проводились: проба СДС, суточное мониторирование электрокардиограммы и артериального давления. ***Результаты.*** Во всех трех группах пациентов регистрировались сопоставимые гипотензивные и антиаритмические эффекты. При назначении небиволола в составе комбинированной терапии у пациентов с ЖНРС на фоне ГБ III стадии или ее сочетания с ИБС отмечалось увеличение диапазона синхронизации и индекса РАС, в отличие от бисопролола и соталола. ***Заключение.*** У пациентов с ЖНРС и ГБ или ее сочетания с ИБС проба СДС позволяет определить оптимальный вариант комбинированной терапии, наиболее позитивно влияющий на функциональное состояние и индекс РАС.

Ключевые слова: регуляторно-адаптивный статус; желудочковые нарушения ритма сердца; бисопролол; небиволол; соталол.

INTRODUCTION

In the context of a modern personalized approach in medicine interest in methods of investigation the body's functional state characterizing global health parameters — quality of life, tolerance to exercise, regulatory-adaptive status (RAS) is expected to increase. [1, 5].

RAS index, integral parameter of cardiorespiratory synchronism (CRS) test considering interaction of the two main vegetative functions — cardiac and respiratory, has been devised and is being introduced into clinical practice for evident based quantitative evaluation of RAS [13]. The test has been devised at the department of normal physiology of Kuban State Medical University under the head of the professor V.M.Pokrovskiy. To record CRS a portable automated system is used (Fig. 1) [6].

The baseline HR (BHR) and synchronization range (SR) parameters — the width of the range, minimal and maximal limits, duration of synchronization development at a minimal and maximal limits (DSD_{min}) are assessed during the test (Fig. 2). After that, on the basis of the data received RAS is calculated using the formula: RAS index = synchronization range width / duration of synchronism development at a maximal level × 100. RAS index of 100 and higher means high regulatory-adaptive reserve, 99–50 — good, 49–25 — satisfactory, 24–10 — low, 9 and lower — unsatisfactory [14].

Findings of clinical investigations of RAS index in healthy individuals and patients with different pathologies have been published in recent years. Differences of RAS index in a person according to the age and gender characteristics, personal peculiar features and temperament are determined. A clear reverse correlation of RAS index and severity of the pathological process have been demonstrated in patients of obstetric-gynecologic, surgical and therapeutic profiles, in clinics of neurology and psychiatry, sports and military medicine [4, 14].

At the same time, scientific literature has it that positive progress of aimed clinical parameters on treatment is not always accompanied by functional parameters improvement [1]. Thus, in patients with chronic heart failure (CHF) without marked sympathicotonia effective therapy with BB metoprolol succinate use led to no RAS improvement [2].

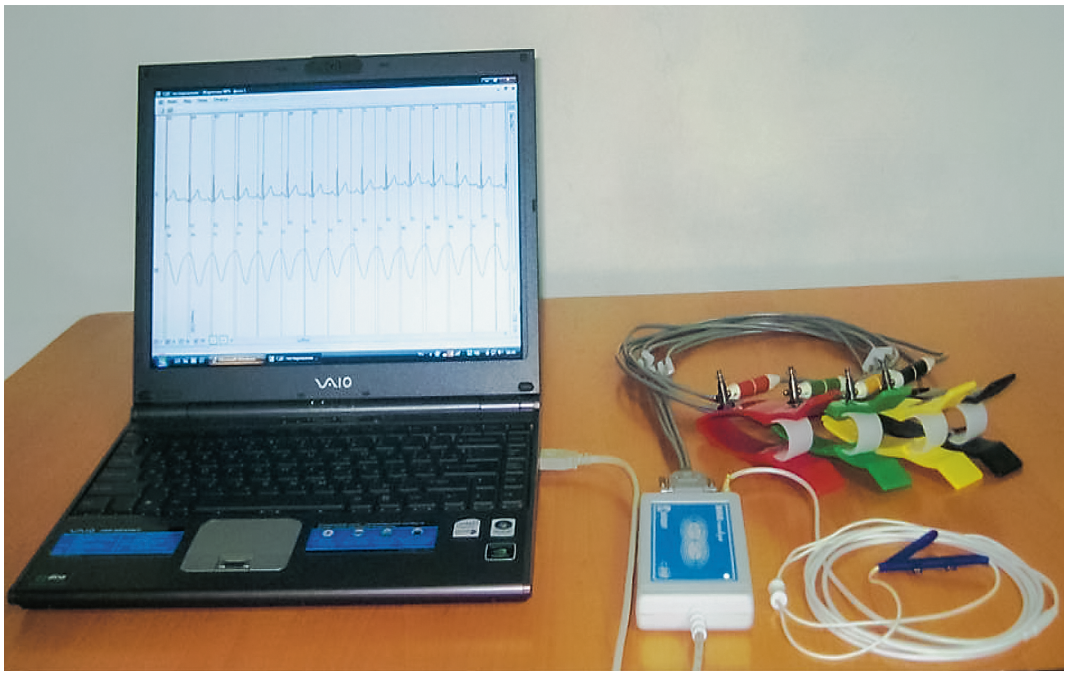

Fig. 1. Portable system for obtaining cardiorespiratory synchronism and analyzing its parameters in humans
Рис. 1. Портативная система для изучения сердечно-дыхательного синхронизма и анализа его параметров у человека

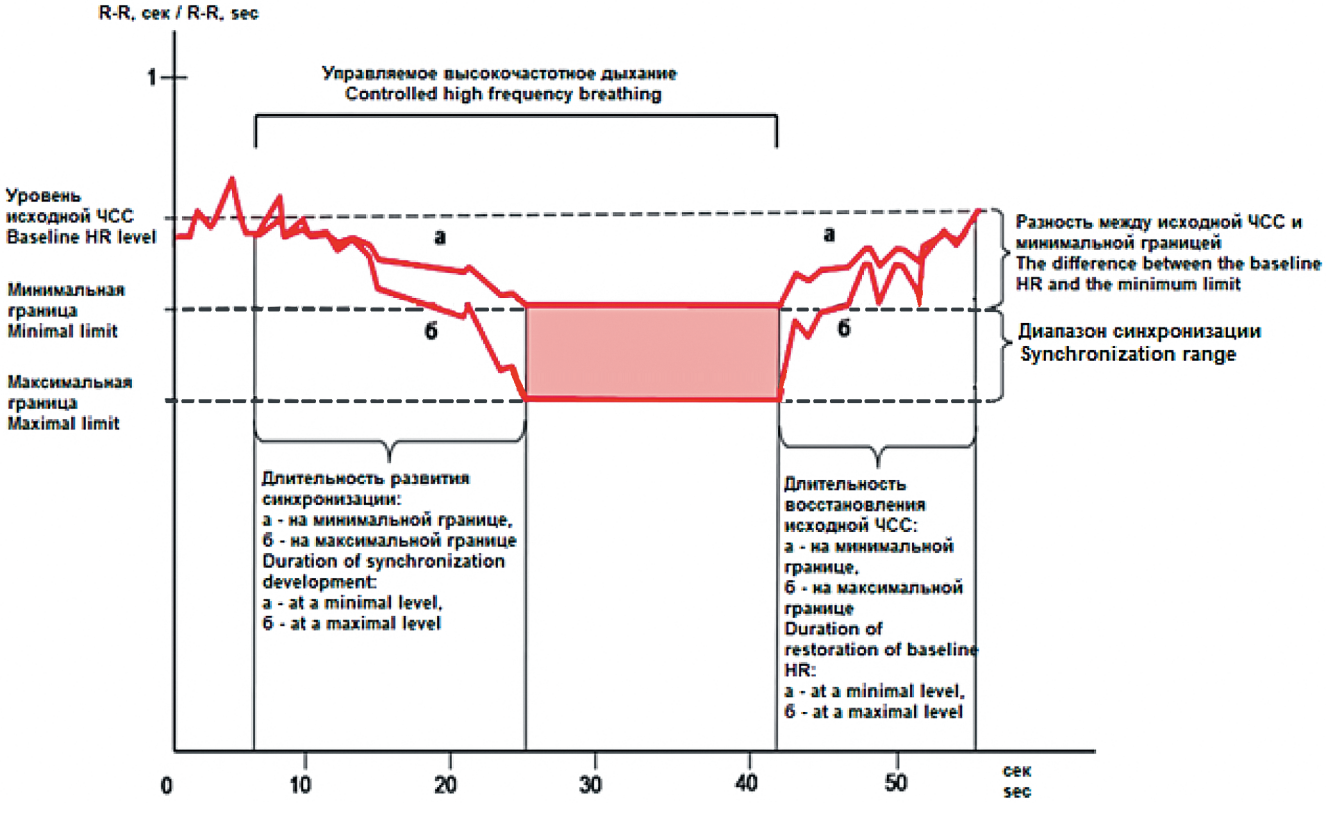

Fig. 2. Indicators of cardiorespiratory synchronism
Рис. 2. Пример показателей сердечно-дыхательного синхронизма

The mentioned phenomenon is likely to be due to specific pharmaceutical drug effect on the affected chains of the vegetative nervous system.

BB are widely used in the routine cardiological practice. Their prescription is most relevant in VA. Ventricular arrhythmias wide spreading and impact on both the prognosis and quality of life explain increased scientific and practical interest in this form of the heart rhythm disorder [8]. Despite the available literature information, currently there is no clearly stated VA treatment strategy. The leading criteria of pharmacotherapy effectiveness is traditionally thought to be the achievement of the antiarrhythmic effects targeted [15]. It is evident that the question of optimal medicine choice should not be reduced to curing arrhythmia. The antiarrhythmic drugs use should provide both achieving clinical effects targeted and positive effect on the functional body state as a whole.

In previous studies reproducibility of CRS and sufficient RAS index responsitivity in patients with VA were shown. And the impact of arrhythmic syndrome etiology and its severity on the RAS index have been demonstrated [7]. However, in modern literature there is no enough evident of BB with different pharmacochemical properties effect on RAS of patients with VA.

OBJECTIVE OF THE STUDY

To study the effect of β-blockers on the parameters of the CRS test in patients with VA.

MATERIALS AND METHODS

The study was carried out on the basis of department of normal physiology of Federal State Budget Educational Institution of Higher Education “Kuban State Medical University” of Ministry of Health of the Russian Federation, department of pathological physiology of Federal State Budget Educational Institution of Higher Education “Military Medical Academy named after S.M. Kirov” of Ministry of Defense of the Russian Federation and department of cardiology of the State Budget Healthcare Institution “Krai clinical hospital № 2” of Ministry of Health of Krasnodar Krai. The study was performed following ethics principles of Declarations of Helsinki [17] and with approval of independent Ethics Committee of FSBEI HE “Kuban State Medical University” of Ministry of Health of the Russian Federation (minute № 65 dated 21.09.2018).

The object of research was 120 people with VA with underlying stage III EH or its combination with coronary heart disease (CHD) who were later randomized into three groups with 40 patients in accordance with antiarrhythmic medicine prescribed as a part of combined therapy: group 1 — bisoprolol, group II — nebivolol and group III — sotalol.

Criteria for inclusion were: age 30–70 years, I–IV grades VA according to B.Lown classification, I–II — according to J.T. Bigger classification, symptomatic hemodynamically insignificant with stage III EH presence and its combination with CHD with safe left ventricle systolic function (ejection fraction ≥50%), without preceding 2 week treatment with prescribed drugs.

Exclusion criteria were: past acute cerebral and cardiac vascular accidents, effort-induced angina pectoris of high grades (III–IV functional classes), severe hypertension (III degree), CHF of high grades (III–IV functional classes) by New-York Heart Association classification and left ventricular systolic dysfunction, contraindications to the medicines being tested, past cardiosurgical and neurosurgical interventions, drug and alcohol abuse, decompensated respiratory, hepatic and renal failure, malignant neoplasms, autoimmune diseases in the exacerbation phase, decompensated endocrine diseases, pregnancy and lactation.

Initial daily dose of bisoprolol and nebivolol is 2.5 mg (a single intake), sotalol — 80 mg (in two divided doses). Daily doses varied with the interval of 14–28 days: bisoprolol and nebivolol — up to 10 mg, sotalol — up to 320 mg (hemodynamic and subjective ecceptability parameters were taken into account). All participants of the study were administered lisinopril, and if necessary atorvastatin in daily dose of 16.1±4.9 mg (n=17), 15.4±4.8 mg (n=15), 15.7±5.1 mg (n=19) and acetylsalicylic acid in daily dose of 94.2±17.7 mg (n=20), 92.8±17.1 mg (n=22), 93.2±15.6 mg (n=12) in the groups correspondingly (Table 1).

Initially and after 24 weeks of treatment a complete physical examination was performed (Table 2).

Statistical processing was made by means of applied program set STATISCICS (version 6.0) and included methods of variation statistics with calculation of arithmetical mean (M), arithmetical mean standard deviation (SD), the t-Student criterion after evaluating the sample according to the Kolmogorov-Smirnov criterion. The differences were considered statistically significant at $p < 0.05$.

RESULTS

In group I on combined therapy with bisoprolol there was a decrease of RAS (according to the CRS test duration of synchronism development at the minimal level of the synchronization range increased by 26.3% ($p < 0.01$), synchronization range decreased by 19.2% ($p < 0.01$) and RAS index decreased by 38.6% ($p < 0.01$)). At the same time antiarrhythmic effects targeted were induced (according to the daily electrocardiogram monitoring the average HR reduced by 21.1% ($p < 0.01$), the number of ventricular premature beats — by 77.3% ($p < 0.05$), number of episodes of ventricular allo-

Table 1

Baseline data of patients with VA included in the study and doses of the main pharmacological agents used (M±SD)

Таблица 1

Исходные данные включенных в исследование пациентов с ЖНРС и дозы основных применяемых фармакопрепаратов (M±SD)

Показатель / Indicator	Группа I / Group I (n=40)	Группа II / Group II (n=40)	Группа III / Group III (n=40)
Возраст (годы) / Ages (years)	53,2±10,8	52,1±12,7	49,8±11,2
Пол (мужчины / женщины) / Gender (men/women)	19 / 21	20 / 20	21 / 19
Длительность ГБ (годы) / Duration of EH (years)	7,1±2,3	6,8±2,0	6,7±2,1
Длительность ИБС (годы) / Duration CHD (years)	4,8±1,2	4,1±1,3	4,5±1,4
ЧСС (в 1 минуту) / HR (in 1 minute)	78,7±9,8	80,2±10,4	81,2±12,3
Артериальное давление: / Blood pressure: / – систолическое / systolic – диастолическое (мм рт.ст.) / diastolic (mm Hg)	 152,1±10,1 98,3±4,1	 158,9±12,2 97,0±4,8	 156,0±10,8 98,6±5,4
БАБ / beta blocker суточная доза (мг в сутки) / daily dose (mg per day)	Бисопролол / Bisoprolol 6,7±1,4	Небиволол / Nebivolol 6,4±2,8	Соталол / Sotalol 166,5±49,1
Лизиноприл / Lisinopril суточная доза (мг в сутки) / daily dose (mg per day)	12,0±4,6	13,5±4,1	14,7±4,5

Table 2

Research methods

Таблица 2

Методы исследования

Метод / Method	Аппарат / Apparatus	Цель исследования / Purpose of the study
Проба СДС [5] / CRS test	ВНС МИКРО (производитель Россия) / VNS MICRO (manufacturer Russia)	Количественная оценка регуляторно-адаптивного резерва / Quantitative assessment of the regulatory-adaptive reserve
Суточное мониторирование электрокардиограммы (СМЭКГ) [11] / Daily electrocardiogram monitoring	МИОКАРД ХОЛТЕР (производитель Россия) / MYOCARD HOLTER (manufacturer Russia)	Контроль антиаритмической эффективности лечения / Monitoring the antiarrhythmic effectiveness of treatment
Суточное мониторирование артериального давления (СМАД) [12] / Daily blood pressure monitoring	BPLab (производитель Россия) / BPLab (manufacturer Russia)	Контроль гипотензивной эффективности лечения / Monitoring the antihypertensive effectiveness of treatment

rhythmia — by 80.5% (p <0.05)). Moreover, according to the daily blood pressure monitoring targeted decrease of the average systolic arterial blood pressure in the day and night time by 21% (p <0.05) and 12,8% (p <0.05) correspondingly and average diastolic blood pressure in the day and night time by 17.9% (p <0.05) and 14% (p <0.05) correspondingly was observed.

In group II a combination of the combined treatment and nebivolol made it possible to improve RAS index (according to the CRS test SR increased by 34.8% (p <0.05), RAS index increased by 27.9% (p<0.05), duration of synchronism development at the minimal level of the synchronization range did not change essentially. At the same time antiarrhythmic effects targeted were induced (according to the daily electrocardiogram monitoring the average HR reduced by 16% (p <0.01), the number of ventricular premature beats — by 72.8% (p <0.05), number of episodes of ventricular allorhythmia — by 79.8% (p <0.05)). Apart from that, according to the daily blood pressure monitoring targeted decrease of the average systolic arterial blood pressure in the day and night time by 25.1% (p <0.05) and 18,2% (p <0.05) correspondingly and average diastolic blood pressure in the day and night time by 18.6% (p <0.01) and 17.1% (p <0.05) correspondingly was observed.

Table 3

Results of the CRS test in patients with VA after 24 weeks of therapy (M±SD)

Таблица 3

Результаты пробы СДС у пациентов с ЖНРС через 24 недели терапии (M±SD)

Показатель / Indicator	Группа I / Group I (n=40)	Группа II / Group II (n=40)	Группа III / Group III (n=40)
ДРСmin / Duration of synchronism development at the minimal level of the synchronization range	17,1±4,2 $p_{I–II}$<0,05 $p_{I–III}$<0,05	13,1±2,8 $p_{II–III}$>0,05	12,8±3,6
ДС / Synchronization range	5,9±1,7 $p_{I–II}$<0,05 $p_{I–III}$>0,05	10,2±2,5 $p_{II–III}$<0,05	6,8±2,0
Индекс РАС / RAS index	34,7±8,4 $p_{I–II}$<0,01 $p_{I–III}$<0,01	77,4±13,6 $p_{II–III}$<0,01	53,1±12,4

Примечание: $p_{I–II}$ — при сравнении показателя между группами I и II; $p_{I–III}$ — при сравнении показателя между группами I и III; $p_{II–III}$ — при сравнении показателя между группами II и III.
Note: $p_{I–II}$ — when comparing the indicator between groups I and II; $p_{I–III}$ — when comparing the indicator between groups I and III; $p_{II–III}$ — when comparing the indicator between groups II and III.

In group III the sotalol use in addition to the combined treatment was accompanied by RAS reduction (in accordance with CRS test there was a decrease of SR by 12.4% (p <0.01), RAS index by 13.7% (p <0.01), and there was no any significant change of duration of synchronism development at the minimal level of the SR). Thus, antiarrhythmic effects targeted were induced (according to the daily electrocardiogram monitoring the average HR reduced by 18.2% (p <0.01), the number of ventricular premature beats — by 77.1% (p <0.05), number of episodes of ventricular allorhythmia — by 80.6% (p <0.05)). Apart from that, according to the daily blood pressure monitoring targeted decrease of the average systolic arterial blood pressure in the day and night time by 24.3% (p <0.05) and 9,1% (p <0.05) correspondingly and average diastolic blood pressure in the day and night time by 19.9% (p <0.01) and 19.9% (p <0.05) correspondingly was observed.

The analysis of the finding progress differences between the groups showed that only in group II RAS status improvement was marked. At the same time, comparable antiarrhythmic and hypotensive effects were recorded in all the groups (Table 3).

DISCUSSION

In this study the comparison of three antiarrhythmic drugs with distinct pharmaco-chemical differences was performed. When studying clinical efficacy of bisoprolol, which belongs to the group of selective lipohydrophil antiarrhythmic medicines of class II, we paid attention to its ability to stabilize cellular membranes. In BIMS, BISOMET, TIBBS, MIRSA studies the efficacy was demonstrated in the prevention of myocardium remodeling in patients with chronic heart failure and a decrease of acute cardiac complications and total mortality in EH and CHD [3, 10].

No less effective lipophilic antiarrhythmic drug of class II is nebivolol with high cardiac selectiveness, which mediates vasodilatory effects due to nitrogen oxide (NO) synthesis by the endothelium. Unlike other BB it does not affect erectile function and also provides fat and carbohydrate metabolism improvement. In clinical projects MR NOED, NEBIS, SENIORS in treatment of EH, CHD and CHF nebivolol reduced total mortality and number of acute cardiac complications, caused left ventricular hypertrophy regressing and controlled arterial hypertension [16].

Sotalol is a hydrophilic nonselective BB presenting the properties of class III antiarrhythmic drugs, as well as the two previous drugs it demonstrated high clinical efficacy. In earlier clinical projects, such as ESVEM, VT-MASS, AVID, sotalol prevented supraventricular cardiac rhythm disorders and ventricular arrhythmias of high grades, provided arterial blood pressure improvement [9].

In this study bisoprolol, nebivolol and sotalol as part of combined therapy demonstrated comparable antiarrhythmic and hypotensive efficacy in patients with VA with underlying stage III EH or its combination with coronary heart disease (CHD), with differences of the drugs effect on the CRS test parameters being revealed. Nebivolol compared to bisoprolol and sotalol increased synchronization range and RAS index. Sotalol compared to bisoprolol increased duration of synchronism development at the minimal level of the synchronization range in a lesser degree, decreased RAS index less significantly.

Currently, there is no overwhelming scientific evidence explaining multidirectional effect of antiarrhythmic medicines on RAS revealed in the work. We suppose that diffe-

rences in the ability to penetrate through hematoencephalic barrier and different pharmacodynamic and pharmacokinetic properties of the mentioned drugs define the direction and degree of changes.

The results obtained require further detailed research within clinical and laboratory studies. The importance of global reserve, adaptive and regulatory reactions is ambiguously interpreted, the role of secondary organ and systemic pathological changes is overestimated. Thus, study of RAS helps estimate functional state of the organism, the impact of pathological process and pharmacotherapy on it that will allow us in future optimize and individualize program of treatment and prevention of cardio-vascular complications in patients of this category.

CONCLUSIONS

1. As part of combination therapy bisoprolol, nebivolol and sotalol caused equal targeted antiarrhythmic and hypotensive effects in patients with VA with underlying stage III EH or its combination with CHD.

2. While administering nebivolol as part of combination therapy patients with VA with underlying stage III EH and its combination with CHD were reported to have increased SR and RAS index compared to bisoprolol and sotalol.

3. The use of CRS test in patients with VA with underlying stage III EH and its combination with CHD makes it possible to determine the most suitable type of combined pharmacotherapy that does not affect RAS.

ADDITIONAL INFORMATION

Author contribution. Thereby, all authors made a substantial contribution to the conception of the study, acquisition, analysis, interpretation of data for the work, drafting and revising the article, final approval of the version to be published and agree to be accountable for all aspects of the study.

Competing interests. The authors declare that they have no competing interests.

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ДОПОЛНИТЕЛЬНАЯ ИНФОРМАЦИЯ

Вклад авторов. Все авторы внесли существенный вклад в разработку концепции, проведение исследования и подготовку статьи, прочли и одобрили финальную версию перед публикацией.

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Источник финансирования. Авторы заявляют об отсутствии внешнего финансирования при проведении исследования.

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CHANGES IN DENTAL HEALTH INDICATORS DURING THE PERIOD OF STUDY IN A MILITARY UNIVERSITY

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Abstract. ***Introduction.*** Currently, the Russian Federation and economically developed countries of the world have achieved a high level of development of aviation technology. At the same time, until today there is no data on changes in the dental health of cadets during their studies at the university, taking into account the factors of military labor. ***The purpose*** of the work — to study the change in the indicators of dental health of cadets during their studies at the university, taking into account the factors of military labor. ***Materials and methods.*** The condition of the organs and tissues of the chewing apparatus was studied in 200 1st-year cadets and 185 graduates of combined-arms military educational institutions and 117 cadets and 111 graduates of military educational institutions for the training of flight personnel to determine the degree of influence of flight work on dental morbidity and its structure, for which an in-depth dental examination was conducted according to generally accepted rules. ***Results.*** Cadets of the 1st year, as well as graduates of combined arms and military educational institutions for the training of flight personnel, as representatives of the same population, had almost the same prevalence and intensity of major dental diseases, as well as the structure of pathology of organs and tissues of the chewing apparatus with a satisfactory level of dental care upon admission to university, and a good level of dental care in the final year university, despite a slight increase in the prevalence and intensity of the course of dental caries and periodontal diseases during the training period, which is associated with the planned sanitation of the oral cavity. Taking into account the high prevalence of inflammatory periodontal diseases (gingivitis, periodontitis) among cadets of the 1st and final courses of the higher military educational institutions (HMEI), dentists serving cadets should, in addition to routine preventive examinations, ensure regular (up to 2 times a year) professional oral hygiene and provide training to the entire serviced contingent of the relevant rules of dental care and the organs of the mouth.

Key words: cadets; military educational institutions; dental health; dental caries; non-carious dental lesions; oral hygiene; periodontal diseases; pathology of the oral mucosa; sanitation; medical examination.

ИЗМЕНЕНИЕ ПОКАЗАТЕЛЕЙ СТОМАТОЛОГИЧЕСКОГО ЗДОРОВЬЯ ЗА ВРЕМЯ ОБУЧЕНИЯ В ВОЕННОМ ВУЗЕ

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Резюме. ***Введение.*** В настоящее время в Российской Федерации и экономически развитых странах мира достигнут высокий уровень развития авиационной техники. В то же время до сегодняшнего дня отсутствуют данные об изменении стоматологического здоровья курсантов за время обучения в вузе с учетом факторов военного труда. ***Цель работы*** — изучить изменение показателей стоматологического здоровья курсантов за время обучения в вузе с учетом факторов военного труда. ***Материалы и методы.*** Изучено состояние органов и тканей жевательного аппарата у 200 курсантов 1-го курса и 185 выпускников общевойсковых военных учебных учреждений и 117 курсантов и 111 выпускников военно-учебных учреждений по подготовке летного состава для определения степени влияния летной работы на стоматологическую заболеваемость и ее структуру, для чего проведено углубленное стоматологическое обследование по общепринятым правилам. ***Результаты.*** Курсанты 1-го курса, а также выпускники общевойсковых и военных учебных учреждений по подготовке летного состава, как представители одной популяции, имели практически одинаковую распространенность и интенсивность основных стоматологических заболеваний, а также структуру патологии органов и тканей жевательного аппарата при удовлетворительном уровне оказания стоматологической помощи при поступлении в вуз, и хороший уровень стоматологической помощи на выпуском курсе вуза, несмотря на незначительное нарастание распространенности и интенсивности течения кариеса зубов и болезней пародонта за период обучения, что связано с проведением им плановой санации полости рта. Принимая во внимание большую распространенность среди курсантов 1-го и выпускного курсов высших военных учебных учреждений (ВВУУ) воспалительных заболеваний пародонта (гингивит, пародонтит), врачи-стоматологи, обслуживающие курсантов, должны, помимо плановых профилактических осмотров, обеспечить регулярное (до 2 раз в год) проведение профессиональной гигиены полости рта и осуществлять обучение всего обслуживаемого контингента соответствующим правилам ухода за зубами и органами рта.

Ключевые слова: курсанты; военно-учебные учреждения; стоматологическое здоровье; кариес зубов; некариозные поражения зубов; гигиена полости рта; заболевания пародонта; патология слизистой оболочки полости рта; санация; диспансеризация.

INTRODUCTION

At present, the Russian Federation and economically developed countries of the world have reached a high level of development of aviation technology. New types of airplanes have appeared, they possess not only large payload, high power and capacity, but also high speed, maneuverability, and “high ceiling” [1, 2]. Obviously, such improvement of aviation equipment leads not only to the relief of pilots’ labor and increase of its efficiency, but also to increased neuroemotional and physical stress, which affects their somatic and dental health [3, 4, 11, 12]. That is why the protection and improvement of health of the flight personnel of the Military Space Forces of the Ministry of Defense of the Russian Federation (MOD RF) and civil aviation is relevant. It is associated with high willingness to combat of the country’s air forces and flight safety [8–10]. It is known that dental diseases occupy one of the leading places in the structure of general morbidity of servicemen of all branches of troops and civilian population [3, 4, 6, 11]. At the same time, until now there are no data on the changes in the dental health of cadets during their higher education that would take into account the factors of military labor.

OBJECTIVE OF THE STUDY

To study the changes in the indicators of dental health of cadets during their higher education taking into account the factors of military labor.

MATERIALS AND METHODS

To implement the ongoing research to study the state of organs and tissues of the masticatory apparatus in cadets and to determine the degree of influence of military labor factors on dental morbidity and its structure, there were examined 200 1st year cadets and 185 graduates of general military educational institutions and 117 cadets and 111 graduates of military educational institutions for training flight personnel. The study included men whose age at the 1st year of study at military educational institutions was 17–22 years, and at their graduation — 22–27 years. It should be noted that graduates of militaryflight personnel training institutions were exposed to aviation flight factors to a greater extent than other similar categories of general military cadets during their training, namely, during training flights and flight practice.

To determine the prevalence and intensity of carious process, periodontal diseases, diseases of the mucous membrane of the oral cavity, tongue and lips (SOPR), masticatory muscles, temporomandibular joint (TMJ), an in-depth dental examination of the cadets was conducted. They were examined using a dental mirror and probe, a special graduated button probe to assess the state of periodontal tissues. The intensity of dental caries was assessed by CFU indices (C — carious teeth; F — filled teeth; U — extracted teeth). The prevalence of dental caries, periodontal and SOPR pathology, as well as the need of personnel in oral cavity sanitation was expressed in percentages. The level of dental care was determined according to the generally accepted method [5]. The hygienic state of the oral cavity (hygiene index) was determined according to Y.A. Fedorov and V.V. Volodkina [5]. Volodkina [5]. Pathologic changes in the periodontium were evaluated using the Schiller–Pisarev test, Svrakov iodine number, and the CFI index (periodontal index) [5], which has proven itself in our earlier epidemiologic surveys of Russian army personnel and the civilian population of the Russian Federation [3, 4, 6].

Based on complaints and objective data of clinical examination (pain sensations in the area of masticatory muscles or TMJ, including palpation data, displacement of the esthetic center of the jaws in the position of central occlusion, presence of mandibular deviation when opening the mouth, etc.), the condition of masticatory muscles and TMJ was evaluated [7].

The digital material obtained in the clinical study was processed on a personal computer using a specialized package for statistical analysis STATISTICA 6.0. Differences between the compared groups were considered reliable at $p \leq 0.05$. Cases when the probability values of the p value were in the range from 0.05 to 0.10 were considered as “presence of a trend”.

RESULTS

It was found that the prevalence of dental caries in 1st-year cadets of general military higher military educational institutions (HMEI) (hereinafter cadets) and 1st-year cadets of flight training HMEI (flight cadets) averaged 89.5 and 87.2% (Fig. 1), and the caries intensity index was 4.66 (C — 1.65; F — 2.35; U — 0.66) and 4.82 (C — 1.95; F — 2.44.9; U — 0.43), respectively ($p \geq 0.05$). Non-carious lesions of teeth in the form of erosion, enamel hypoplasia and wedge-shaped defects were equally frequent in both groups of patients, in 11.5 and 7.7% of cases, respectively ($p \leq 0.01$). Pathologic erasability of the hard tissues of teeth was not diagnosed in any of the examined patients. However, while 70% of the cadets needed treatment for dental hard tissue pathology, this figure was 65.0% ($p \leq 0.05$) in the flight cadets, and the level of dental care (LDC) in both groups was evaluated as satisfactory (Fig. 2), and the numerical expression of the LDC index was 55.6 and 51.0% in the above groups, respectively ($p \geq 0.05$).

The hygienic state of the oral cavity assessed by the hygiene index (HI) of Y.A. Fedorov–V.V. Volodkina had no significant differences in all examined groups of 1st year cadets. The level of oral hygiene was considered as unsatisfactory (IG was 1.90–1.91).

When assessing the state of periodontal tissues, gingival bleeding (positive Ainamo test) and positive Schiller–Pisarev test were detected in 66.5% of 1st year cadets and 65.8% of 1st year flight cadets with Svrakov iodine number of 0.83±0.11 and 0.69±0.11 units, respectively ($p \leq 0.05$), which indicated the presence of gingivitis and required mandatory professional oral hygiene.

Supragingival and (or) subgingival tartar deposits were diagnosed in the studied groups in 18.5 and 16.2% of cases, respectively ($p \geq 0.05$). At the same time, periodontal pockets up to 5 mm deep were found in 6.5% of cadets and 5.1% of flight cadets ($p \geq 0.05$). This category of subjects (Fig. 3) certainly needed comprehensive treatment of periodontitis.

It should be emphasized that cadets and cadets of flight in the 1st course were mostly diagnosed with mild (in single cases — of medium intensity) periodontal diseases. The KPI index in the studied groups respectively amounted to 1.91±0.21 and 1.90±0.22 units ($p \geq 0.05$). The dystrophic form of periodontal disease was not diagnosed in any of the examined patients.

In both groups, diseases of the oral mucosa, lips and tongue were rarely detected (Fig. 3), respectively in 2.0 and 1.71% of cases ($p \geq 0.05$). Among these diseases were glossitis (folded, “geographic” tongue), chronic recurrent herpetic stomatitis, and meteoric cheilitis.

In 2.0% of the 1st year cadets and 1.71% of the 1st year PLS cadets various pathological symptoms from the TMJ side were determined, which allowed to diagnose the presence of TMJ dysfunction, and in 50% of them this pathology was combined with parafunction of masticatory muscles (bruxism).

Obviously, from the data obtained, we can conclude that cadets entering military educational institutions, regardless of the military specialty of the higher educational institution, require full-fledged dental rehabilitation, which should and can be realized within the scheduled oral cavity sanation of these groups of servicemen.

The study has shown that caries prevalence in cadets — graduates of general military higher military educational institutions (hereinafter referred to as graduates) amounted to 92.4%, and in graduates of flight training in general military higher military educational institutions (hereinafter referred to as graduates of flight trainig) — 91.0%, the intensity of

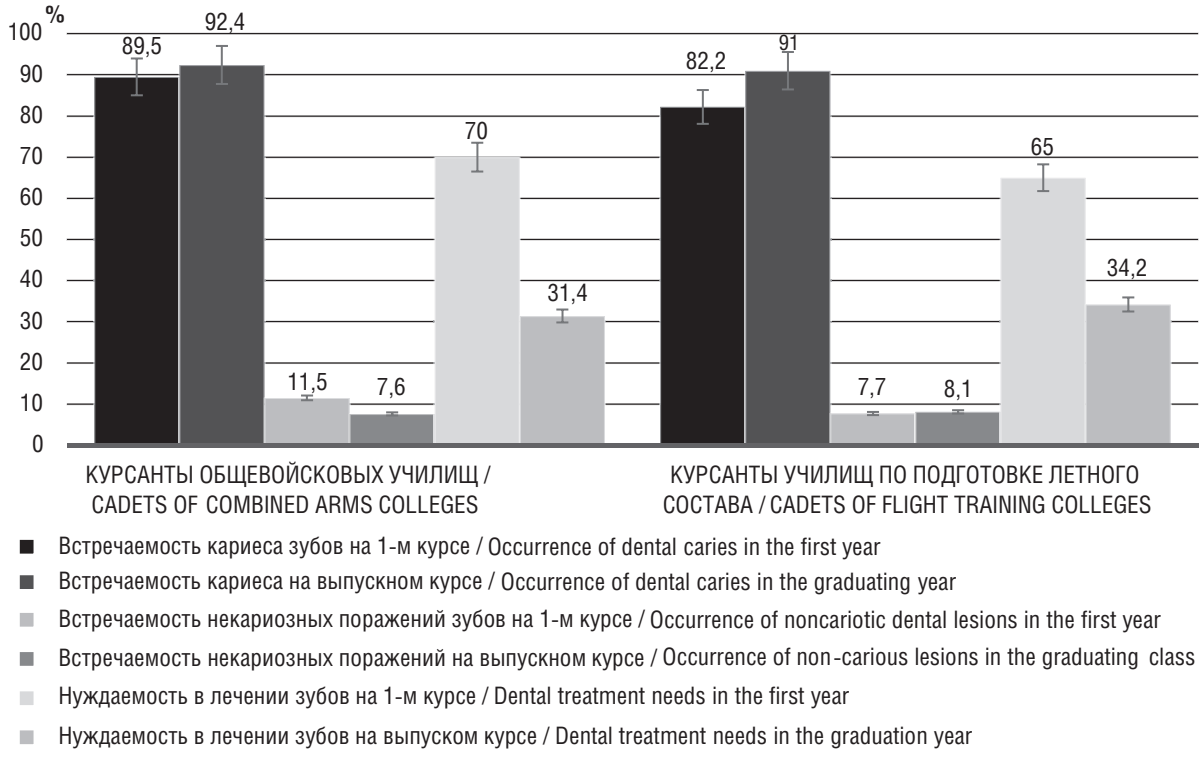

Fig. 1. The occurrence of caries and non-carious teeth and the need for their treatment in those examined in the first and final year of a military educational institution (%)

Рис. 1. Встречаемость кариеса и некариозных зубов и нуждаемость в их лечении у обследованных на 1-м и выпускном курсе военно-учебного учреждения (%)

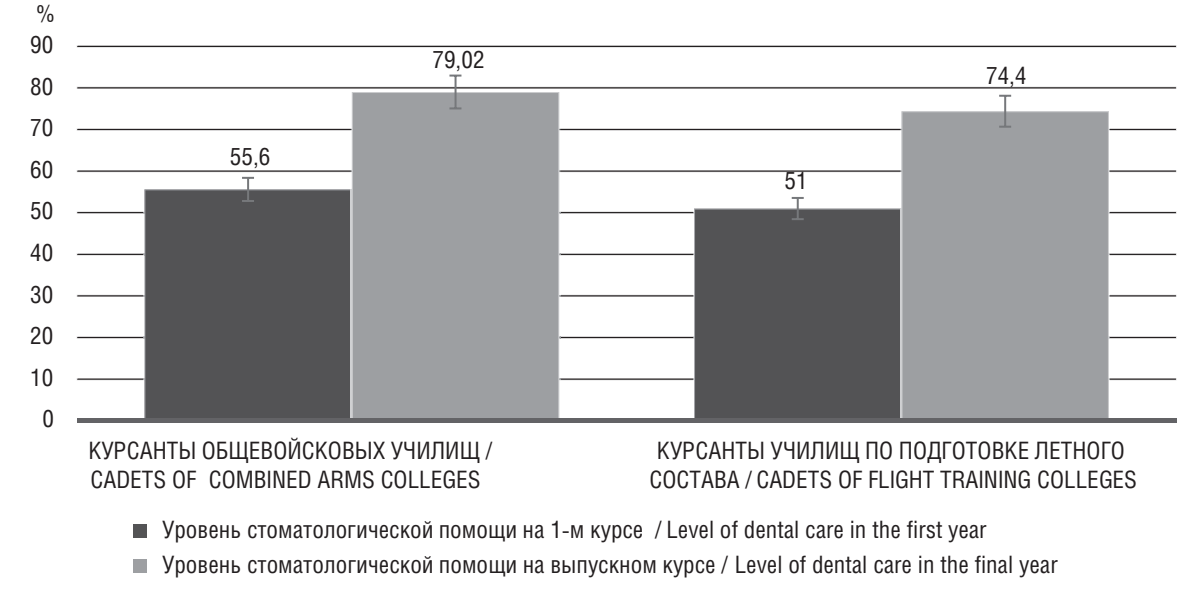

Fig. 2. The indicator of the level of dental care for those examined in the first and final year of a military educational institution (%)

Рис. 2. Показатель уровня стоматологической помощи у обследованных на 1-м и выпускном курсе военно-учебного учреждения (%)

caries — the CFU index — was 6.2 (C — 0.9; F — 4.7; U — 0.6) and 6.34 (C — 1.12; F — 4.6; U — 0.62), respectively. According to the World Health Organization (WHO) grid, the caries incidence in graduates, as well as in 1st year cadets, should be assessed by prevalence as massive, and by intensity as high. The study of the caries course dynamics during the period of training in the HMEI allowed to reveal some increase in the prevalence and intensity of the

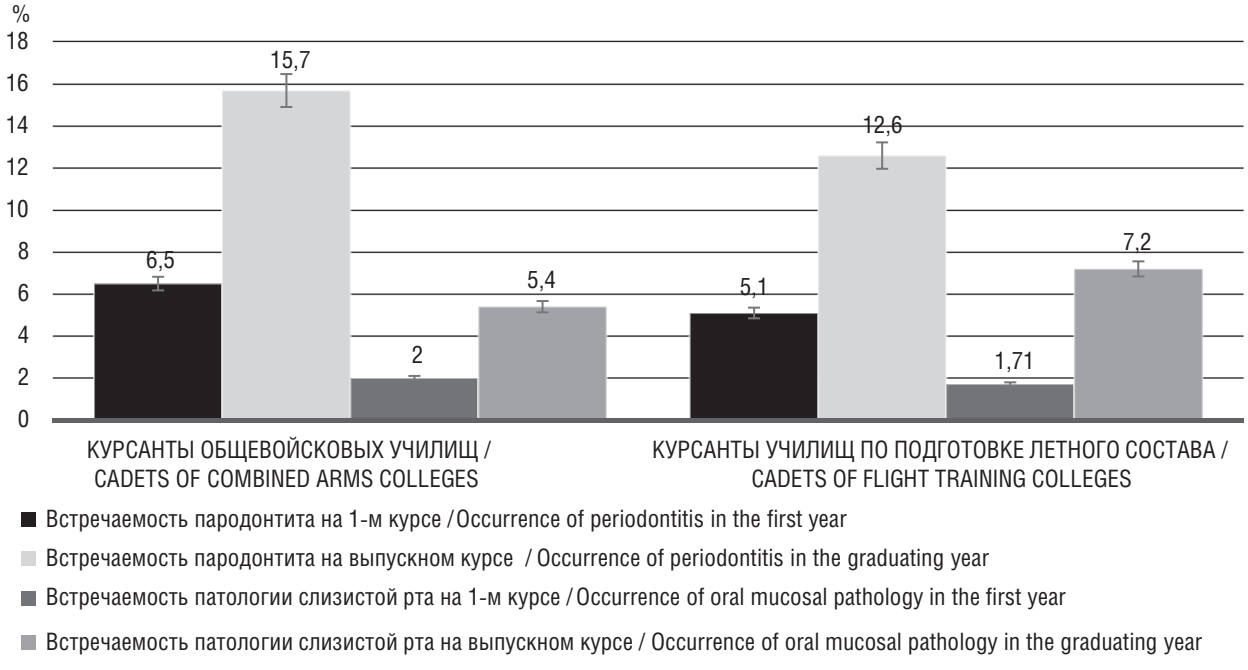

Fig. 3. Occurrence of periodontitis and pathology of the oral mucosa in the examined (%)
Рис. 3. Встречаемость пародонтита и патологии слизистой оболочки рта у обследованных (%)

carious process regardless of the HMEI profile (p ≥0,05). The prevalence of non-carious tooth lesions in both groups of graduates was 7.6 and 8.1%, respectively (p ≥0.05). It is important to emphasize that due to medical examination and dental therapeutic and preventive measures in HMEI, 31.4% of graduates and 34.2% of PLS graduates needed treatment of dental diseases, and the level of dental care (LDC) in both groups was assessed as good. The LDC index scores were 79.03 and 74.4%, respectively (p ≤0.01, compared to those in the 1st year of HMEI).

There were significant changes in the state of periodontal tissues in graduates of both groups. The incidence of periodontitis increased according to age in both groups. The level of oral hygiene remained unsatisfactory (HI was 1.9). When evaluating the condition of periodontal tissues, gingival bleeding and positive Schiller-Pisarev test were detected in 40.2% of graduates and 44.9% of flight graduates, respectively. The Svrakov iodine number in these groups were 1.21±0.11 and 1.31±0.17 units, respectively (p ≥0.05). Dental tartar deposits (supragingival and/or subgingival) were diagnosed in 46.5 and 38.7% of the examined graduates and flight training graduates, respectively. At the same time, periodontal pockets up to 5 mm deep were found in 15.7% of graduates and 12.6% of PLS graduates (p ≤0.05). It should be emphasized that all graduates of both study groups were mostly diagnosed with mild intensity of periodontal diseases — CFU index = 1.64±0.22 (CPU index ranged from 1.2 to 2.4). The dystrophic form of periodontal disease was not diagnosed in any examined person.

The diseases of the oral mucosa, tongue and lips also became more frequent in graduates and PLS graduates — in 5.4 and 7.2% of cases, respectively. Among this pathology, herpetic stomatitis, cheilitis, fissure of the red border of the lower lip and glossitis (folding and desquamative tongue) were more frequent.

The prevalence of TMJ disorders in graduates was 2.1% and 1.8% in PLS graduates who simultaneously suffered from masticatory muscle pathology, namely teeth grinding (bruxism).

DISCUSSION

Graduates of general military higher military educational institution (HMEI) and HMEI flight trainig graduates represent the same population and have similar prevalence and intensity of major dental diseases. We could not notice any influence of flight work factors on the masticatory apparatus of flight trainig graduates of HMEI. Despite the insignificant increase in the prevalence and intensity of dental caries during the period of training at HMEI, both groups have a good indicator of dental care level, which can be explained by the most advanced form of dental care (medical checkup) used in the Armed Forces of the Russian Federation for all groups of servicemen. At the same time, periodontal diseases are more frequent and intensive in HMEI graduates, which requires their complex treatment. The unsatisfactory hygienic condition of the oral

cavity of cadets and graduates of the HMEI, regardless of their specialty, plays a certain important role in this, which determines the high prevalence of inflammatory periodontal diseases (gingivitis, periodontitis) among them. It also requires regular training of the entire institution body under examination in the appropriate rules of dental and oral cavity care and professional oral hygiene.

CONCLUSIONS

1. As a result of the planned sanation work in HMEI, the 1st year cadets, as well as graduates of general military and flight schools, as representatives of one population, have practically the same prevalence and intensity of the main dental diseases, as well as the structure of pathology of organs and tissues of the masticatory apparatus, with a satisfactory level of dental care on admission, and a good level of dental care on graduation.

2. The volume and quality of dental work among young people of pre-conscription and conscription age does not correspond to the modern requirements. In this regard, we can predict a sharp change in the structure of dental morbidity among military personnel towards increasing the intensity of caries and its complicated forms, as well as increasing the prevalence and intensity of inflammatory periodontal diseases. This should be taken into account when drawing up the sanation calendar plan in HMEI.

3. The implementation of regular (up to 2 times a year, combined with preventive examinations) professional controlled oral hygiene and dental hygiene training in the structure of dental care will reduce the prevalence of inflammatory periodontal diseases (gingivitis, periodontitis) among cadets and trainees of HMEI.

ADDITIONAL INFORMATION

The author read and approved the final version before publication.

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Consent for publication. Written consent was obtained from the patient for publication of relevant medical information within the manuscript.

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Автор прочитал и одобрил финальную версию перед публикацией.

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EVALUATION OF THE GENUS *CANDIDA* FUNGI ADHESIVE PROPERTIES ON THE MATERIALS USED IN DENTISTRY

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Abstract. ***Introduction.*** Acrylamide plastics are widely used in orthopedic dentistry. Studies of their susceptibility to microbial adhesion are relevant, since the restorative material made on the basis of these polymers can become a reservoir for microorganisms that can infect peri-implant tissues and cause inflammation. ***The purpose of the study*** was to test the adhesive ability of clinical strains of *Candida* to samples of acrylamide plastics. ***Materials and methods.*** 50 clinical strains of *Candida* fungi have been studied for the formation of biofilms during cultivation on acrylamide plastics. Plastic samples were treated with an inoculum of fungal cultures for 48 h at 37 °C. The values of their optical density (λ=560 nm) were a quantitative assessment of the biomass of the formed films. ***The results of the study.*** Quantitative analysis of the biofilm biomass showed that after 48 h all fungal strains formed a biofilm on the surface of the tested polymer discs. The highest quantitative values of the biofilm biomass were noted in the cultivation of *C. albicans.* ***Conclusion.*** It was noted that the type of material is not a key growth restriction factor for *C. albicans*. A set of measures is needed that combines optimal mechanical processing methods with the use of antimicrobial drugs to prevent the formation and accumulation of biofilms.

Key words: fungi of the genus *Candida*; *Candida albicans*; biofilms; acrylamide plastics.

ОЦЕНКА АДГЕЗИВНЫХ СВОЙСТВ ГРИБОВ РОДА *CANDIDA* НА МАТЕРИАЛАХ, ИСПОЛЬЗУЕМЫХ В СТОМАТОЛОГИИ

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Резюме. ***Введение.*** Акриламидные пластмассы широко используются в ортопедической стоматологии. Исследования их восприимчивости к микробной адгезии актуальны, поскольку изготовленный на основе этих

полимеров реставрационный материал может стать резервуаром для микроорганизмов, которые могут поражать периимплантные ткани и вызывать воспаление. ***Целью исследования*** было проверить адгезионную способность клинических штаммов грибов рода *Candida* к образцам акриламидных пластмасс. ***Материалы и методы.*** *И*сследовано 50 клинических штаммов грибов рода *Candida* на предмет образования биопленок при культивировании на акриламидных пластмассах. Образцы пластмасс обрабатывали инокулятом культур грибов в течение 48 ч при 37 °С. Количественной оценкой биомассы сформировавшихся пленок были значения их оптической плотности (λ=560 нм). ***Результаты исследования.*** Количественный анализ биомассы биопленки показал, что через 48 ч все штаммы грибов образовали биопленку на поверхности тестируемых полимерных дисков. Самые высокие количественные значения биомассы биопленок были отмечены при культивировании *C. albicans*. ***Заключение.*** Было отмечено, что тип материала не является ключевым фактором ограничения роста для *C. albicans*. Необходим комплекс мероприятий, сочетающий оптимальные методы механической обработки с использованием антимикробных препаратов для предотвращения образования и накопления биопленок.

Ключевые слова: грибы рода *Candida*; *Candida albicans*; биопленки; акриламидные пластмассы.

INTRODUCTION

The oral cavity is the most important biotope of the human body which is inhabited by various microflora represented by more than 700 kinds of the microorganisms and which plays an important role in cooperation of the human body with its environment [6]. A specific microbiota inhabits the oral cavity and tends to colonize the surfaces of the teeth, tongue and mucous membranes of the mouth, soft tissues, tooth implants and restorative materials.

The microorganisms colonizing the oral cavity mainly remain in the biotopes where the biofilm formation takes place. As a part of biofilms the microbes become more resistant to the immune factors, antibiotics etc. instead of those solely inhabiting ones, this factor promotes and aggravates such oral conditions as caries, periodiodontal disorders, infections associated with the implants and candidosis of oropharynx. Polymicrobial biofilm infection on the teeth implants is considered to be the main cause of periimplant diseases. Oral streptococci such as *Streptococcus sanguinis, Streptococcus mutans, Streptococcus oralis* and *Streptococcus mitis* are considered to be the colonization "pioneers" prior to plague formation [11], the process involves both the bacteria and the fungi. Thus the oral cavity is being colonized by various genus *Candida* fungi which are commonly associated with the disorders of the mucous membranes of the oral cavity. *Candida albicans*, *Candida tropicalis, Candida parapsilosis, Candida glabrata, Candida krusei* and *Candida dubliniensis* are most common types among them antibiotic-resistant strains are present [1, 4]. It is revealed that genus *Candida* fungi are characterized by expressive adhesive properties and they can be absorbed in biotic and abiotic surfaces including acrylic dentures. Adhesion and colonization seem the first and the most important stage in infectious process underlying and preceding the biofilm formation which involves not only the mucous membranes but also the surfaces of the medical devices [3], thus, resulting in infection and the following medical assistance. Nowadays the genus *Candida* fungi ability to produce biofilms is considered as the most important factor of virulence [5], resulting in grave clinical conditions. Genus *Candida* fungi present in biofilms is highly resistant to antifungal therapy and resists to some immune factors of the host.

OBJECTIVE OF THE STUDY

The purpose of the study was to test the adhesive ability of clinical isolates of *Candida* strains to samples of acrylamide plastics which is used in prosthetics.

MATERIALS AND METHODS

Methyl methacrylate plastics Belacryl-M HO (Russia), ethyl methylmethacrylate plastics Belacryl-E HO (Russia) and Protacryl-M material (Ukraine) samples were investigated. 10 samples from each material with the diameter of 20 mm and height of 8 mm were involved. 50 strains of Candida fungi were studied: *C. albicans* — 23, *C. tropicalis* — 14, *C. krusei* — 8, *C. glabrata* — 5. All the strains were cultivated in 5% blood agar (24 h at 37 °C) to obtain separate colonies. To prepare inoculum the cultures were suspended in fluid Biomedia (Saburo) environment until they reached the density of D_{600} 0,025±0,005 (NanoPhotometer N60-Touch, Germany). The tested materials were placed in the plates (Fudau Biotechnology, Russia) were 2 ml of inoculum were introduced. Cultivation lasted 48 hours at 37 ° C. After incubation the samples were thoroughly washed twice in phosphate buffered saline (PBS), pH 5.0, to remove planktonic cells. The biofilm biomass was assessed by the method [2] according to the research modi-

Table 1

Quantitative characteristics of biofilm biomass based on absorption values depending on the type of restoration material

Таблица 1

Количественные характеристики биомассы биопленки на основе значений поглощения в зависимости от типа реставрационного материала

Материал / Material	*C. albicans*	*C. glabrata*	*C. tropicalis*	*C. krusei*
Белакрил-М ХО / Belacril-M HO	2,62	1,29	1,54	1,9
Белакрил-Э ХО / Belacril-E HO	2,07	1,43	1,8	1,54
Протакрил-М / Protacril-M	2,19	2,0	2,04	1,62

Table 2

Number of viable microorganisms in biofilms, expressed in colony forming units (CFU/ml)

Таблица 2

Количество жизнеспособных микроорганизмов в биопленках, выраженное в колониеобразующих единицах (КОЕ/мл)

Материал / Material	*C. albicans*	*C. glabrata*	*C. tropicalis*	*C. krusei*
Белакрил-М ХО / Belacril-M HO	$5,87\times10^6$	$1,63\times10^6$	$2,28\times10^6$	$2,86\times10^6$
Белакрил-Э ХО / Belacril-E HO	$5,14\times10^6$	$1,80\times10^6$	$2,62\times10^6$	$2,24\times10^6$
Протакрил-М / Protacril-M	$5,99\times10^6$	$3,05\times10^6$	$3,14\times10^6$	$2,24\times10^6$

fication: the biofilm stained by gentian violet was extracted with ethanol, decanted, and separated 20 times to assess the optical density by spectrophotometer (PE-5400 UV) at a wavelength of 560 nm. Sterile disks were used as a negative control.

The amount of viable microorganisms in the biofilm was detected by counting colony forming units (CFU). After biofilm formation the samples were thoroughly washed three times in 1 ml of PBS to remove the cells which were not detached. Then they were placed in centrifuge tubes filled with 1 ml of PBS, they were vigorously shaked for 2 min in LAUDA Varioshake VS 15 R (Germany) to disperse the cells which sticked to the disk surface. The cellular suspension of each sample was three times consequently dissolved in PBS ten times and applied Saburo agar. They were incubated for 48 h at 37° C. The amount of the viable cells which formed the colonies was indicated in CFU/mL. All the results are represented statistically according to STATISTICA 12.0.

RESULTS AND DISCUSSION

The quantitative analysis of the biomass formed on the biofilm revealed that all strains of the fungi formed the biofilm on the surface of the tested polymer disks in 48 h. The quantitative analysis noted the highest number of biofilm biomass represented by of *C. albicans* (Table 1).

High values of biofilm biomass in cultivation of *C. albicans* to a great extent are associated with the adhesive activity of these fungi. In comparison with the other *Candida* genus, *C. albicans* forms complicated biofilms which are composed of a merging basal layer of blastospores and covered with thick layer of matrix and containing extracellular material and hypha. The other isolates form only blastospore basal layer [8;10]. Morphological transition of *C. albicans* from yeast to micelial cells is one of the main factors of virulence as it contributes not only to significant spread of the culture on the polymer surface but also impairment of the mucous membrane of the oral cavity caused by acid proteases.

The results of calculation of the viable cells derived from the biofilms referred to the dynamic of biofilm biomass formation by different genera of *Candidae* (Table 2).

It was noted that the type of the material was not a limiting key factor for *C. albicans* growth. Though there was decrease of *C. glabrata* and *C. tropical* growth on the surface of Belacryl-M HO.

Survival rate of *C. albicans* on polymer materials is associated with extracellular polymer matrix formation ability, which overlaps the cells and pseudohyphae of the fungi, thus, it prevents the inhibiting factors [12].

Acrylamyd plastics are the most common type of polymer materials in dentistry. Nevertheless there are no extended research works concerning biofilm formation on these materials. Microorganisms which adhere to the dental implants and dentures can cause different infectious processes, aggravating pulp pathology in particular [7, 9]. Intensive biofilm formation on the restorative materials in the oral cavity

is revealed in a day, even a temporary restorative material can aggravate the oral health condition, which requires measures to prevent accumulation of the microorganisms and formation of the biofilm.

CONCLUSION

All the investigated genus *Candida* types have shown high capability in biofilm production in all mentioned above acrylamide plastic samples. It was revealed that *Candida albicans* was characterized by the highest quantitative value prevalence. Intensive biofilm formation by the fungi on these materials during the restorative work in the oral cavity is a risk factor for infectious complications. Complex of measures including optimum mechanical debridement in combination with antimicrobial medications are required for effective oral hygiene.

ADDITIONAL INFORMATION

Author contribution. Thereby, all authors made a substantial contribution to the conception of the study, acquisition, analysis, interpretation of data for the work, drafting and revising the article, final approval of the version to be published and agree to be accountable for all aspects of the study.

Competing interests. The authors declare that they have no competing interests.

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Вклад авторов. Все авторы внесли существенный вклад в разработку концепции, проведение исследования и подготовку статьи, прочли и одобрили финальную версию перед публикацией.

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Источник финансирования. Авторы заявляют об отсутствии внешнего финансирования при проведении исследования.

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CHARACTERISTICS OF CHONDROPLASTIC MATERIALS: ADVANTAGES AND DISADVANTAGES

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Abstract. ***Introduction.*** Articular cartilage, due to the peculiarities of its structure and the lack of active trophism, is not capable of independent regeneration. Existing clinical methods for cartilage tissue restoration have many limitations. The development of tissue-engineered structures remains an urgent task in the fields of medicine, biology, and materials science. ***Purpose of the study:*** to analyze existing materials for chondroplasty and identify their advantages and disadvantages. ***Materials and methods.*** The study design was a non-systematic literature review. The data search was carried out in the following databases: PubMed, ScienceDirect, eLibrary, Google Scholar. The search period was 15 years; most of the works included in the study were published in the last 5 years. Criteria for inclusion of works: availability of the full text of the articles, availability of histological studies, availability of statistical data analysis. The exclusion criteria for works were the absorbing nature of articles by one author (a more recent publication was included in the analysis). ***Results.*** During the work, it was found that both biological and synthetic polymers are used in the development of chondroplastic materials. Biological polymers have a high affinity for cell cultures but are not able to withstand significant mechanical loads. The solution of mechanical strength is the use of synthetic polymers. Chondrocytes are used as the main cell culture that influences the acceleration of defect restoration. Differentiation factors, especially factors from bone morphogenetic proteins group (BMPs), are also actively used. ***Conclusion.*** Biopolymers and synthetic polymers have both advantages and disadvantages, which leads to the need to use different types of polymers to ensure the mimicry properties of the structures being developed. The use of growth factors, differentiation factors, cell cultures and biologically active substances accelerate regeneration processes.

Key words: chondroplasty; tissue engineering; biological polymers; chondrocytes.

ХАРАКТЕРИСТИКА ХОНДРОПЛАСТИЧЕСКИХ МАТЕРИАЛОВ: ПРЕИМУЩЕСТВА И НЕДОСТАТКИ

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Резюме. ***Введение.*** Суставной хрящ, ввиду особенностей своего строения и отсутствия активной трофики, не способен к самостоятельной регенерации. Существующие клинические методы восстановления хрящевой ткани имеют множество ограничений, из-за чего разработка тканеинженерных конструкций остается актуальной задачей в области медицины, биологии и материаловедения. ***Цель исследования:*** провести анализ существующих материалов для хондропластики и выявить их преимущества и недостатки. ***Материалы и методы.*** Дизайн исследования представлен несистематическим обзором литературы. Поиск данных осуществляли в базах данных PubMed, ScienceDirect, eLibrary, Google Scholar. Глубина поиска составила 15 лет, большинство работ, включенных в исследование, опубликованы в последние 5 лет. Критерии включения работ: наличие полного текста рукописи, наличие гистологических исследований, статистического анализа данных. Критериями исключения работ считали: поглощающий характер статей одного автора (в анализ включали более позднюю публикацию). ***Результаты исследования.*** В ходе работы было установлено, что в разработке хондропластических материалов применяются как биологические, так и синтетические полимеры. Биологические полимеры обладают высоким сродством к культурам клеток, при этом не способны выдерживать значительные механические нагрузки. Решением проблемы механической прочности является применение синтетических полимеров. В качестве основной культуры клеток, которая влияет на ускорение восстановления дефекта, используются хондроциты. Активное применение находят также дифференцировочные факторы, в особенности факторы из числа костных морфогенетических белков (BMP). ***Заключение.*** И природные биополимеры, и синтетические полимеры имеют как преимущества, так и недостатки, что приводит к необходимости применения разных типов полимеров для обеспечения мимикрических свойств разрабатываемых конструкций. Применение факторов роста, факторов дифференцировки, клеточных культур и биологически активных веществ способствует ускорению процессов регенерации.

Ключевые слова: хондропластика; тканевая инженерия; биологические полимеры; хондроциты.

INTRODUCTION

The treatment of traumatic injuries and degenerative changes in articular cartilage represents one of the challenging tasks in the practice of orthopedic traumatologists. Articular cartilage (AC) is a unique connective tissue that plays a pivotal role in maintaining joint mobility by reducing mechanical friction at joint surfaces and absorbing shock during load transmission.

The absence of vascularization and innervation, the limited number of progenitor cells, and the restricted proliferative potrntial of mature chondrocytes contribute to the inability of cartilage tissue to repair itself. The currently employed methods of cartilage tissue transplantation, as well as subchondral bone transplantation, offer numerous clinical benefits but also possess drawbacks such as insufficient material availability, immunogenicity risks, and the complex preparation of implantable samples. Synthetic and natural biopolymers, which are devoid of these limitations, may be considered an alternative. Moreover, complex polymer compositions in conjunction with cells, growth factors, and differentiation factors can be used for cartilage tissue engineering. Biocompatibility, an extended resorption period, the enhancement of chondrogenesis, and the replication of the extracellular matrix structure of cartilage tissue are the main requirements for synthetic cartilage transplants.

Currently, there is no unified classification for articular cartilage damage across different localizations. The most widely used classifications in clinical practice are those proposed by Outerbridge [60] and by Bauer and Jackson [9]. The most comprehensive classification is that proposed by the International Cartilage Repair Society (ICRS) in 2000. Each of these classifications is based on the histomorphological changes in articular cartilage, characterized by the stage of progression or the severity of the damage. In describing the state of AC, the size of the defect as well as its anatomical and functional localization are also taken into account."

A great number of publications in the unit "Clinical Medicine are devoted to the epidemiologically most significant dis-

eases that lead to the development of degenerative-dystrophic changes in AC. Among the primary causes of osteoarthritis associated with autoimmune-driven pathogenesis are juvenile idiopathic arthritis and ankylosing spondylitis. Researchers also note that high doses of glucocorticosteroids used in the therapy of such conditions, as well as alterations in the composition and volume of synovial fluid, play a part. There have also been reports on osteochondritis dissection of the femoral condyle, or König's disease [15, 71]. Legg–Calvé–Perthes disease is recognized as the most frequent condition leading to hip osteoarthritis, attributed to the development of multiplanar deformities in the proximal femur and joint decentration. The ischemic component of this disease's pathogenesis may be associated with the subsequent degeneration of the femoral head's articular cartilage, with progression to deforming osteoarthritis consistently documented in multiple studies. [56, 62]. Limited research has focused on pediatric osteochondropathies, such as Osgood–Schlatter and Blount's disease, various forms of epiphyseal dysplasia, and congenital or acquired lower limb deformities. When these conditions persist with an aggressive course, they can lead to incongruence of the articular surfaces, resulting in uneven load distribution on the cartilage that, in turn, causes its thinning and degenerative changes [4].

The present work provides an analysis of the advantages and disadvantages of the materials most frequently used in cartilage tissue engineering. The article discusses methods to improve cell adhesion and proliferation, as well as the application of composite formulations designed to overcome the shortcomings of the scaffolds under review.

MATERIALS AND METHODS

The study design is presented as a non-systematic literature review. Data were searched in the following databases: PubMed, ScienceDirect, eLibrary, and Google Scholar. The search spanned a period of 15 years, with most of the works included in the study published within the last 5 years. The inclusion criteria were as follows: availability of the full text of the manuscript, the presence of histological studies, and the inclusion of statistical data analysis. Studies were excluded if they represented repetitive work by the same author (only the most recent publication was included in the analysis).

RESULTS AND DISCUSSION

Cartilage tissue — a special type of connective tissue distinguished by its dense and elastic extracellular matrix. Three types of cartilage are recognized:

- Hyaline cartilage — a translucent cartilage tissue with a high content of collagen fibers; it forms the articular surfaces of long bones as well as the edges of the ribs.
- Elastic cartilage — yellowish in appearance due to the presence of elastic fibers; it forms the auricle and the laryngeal cartilages.
- Fibrocartilage — a variant of hyaline cartilage that contains numerous bundles of collagen fibers; fibrocartilage forms intervertebral discs and serves as the attachment sites for tendon–muscle fibers to bones.

Articular cartilage is a type of hyaline cartilage that covers the epiphyses of bones and serves as an interlayer between them. AC is composed of collagen fibers and chondrocytes, which are spherical cells with an average diameter of 13 μm [44]. Chondrocytes constitute 5–10% of the cartilage volume, and their primary role is the formation of the extracellular matrix (ECM), which consists of collagen and proteoglycans. The matrix also contains a large amount of water with dissolved sodium, chloride, and potassium ions. In addition to its joint function, the ECM serves as a barrier protecting the chondrocytes from damage [34].

Articular cartilage is entirely devoid of nerve endings and vascular structures. Chondrocytes are nourished by diffusion from the synovial fluid. The lack of direct nourishment and innervation does not allow cartilage tissue to recover independently, which is why the development of highly effective materials for the repair of articular surfaces remains a critical challenge in medicine [50].

In addition to its protective function for the bone epiphyses, articular cartilage also performs an amortizing role; the presence of synovial fluid and its smooth surface reduce friction in the joints during movement, thereby ensuring the congruence of the articular surfaces. Despite these adaptive features, AC remains vulnerable to degeneration under various stressors.

MATERIALS FOR CARTILAGE REPAIR

The aforementioned histological and morphological features of hyaline cartilage, along with the multitude of pathologies that lead to its damage, underscore the need to develop materials that can restore and replace the lost cartilage volume.

Materials for chondroplasty — whether for complete or partial restoration of articular cartilage in surgery — can be classified into the following groups: biological materials (including autografts and allografts, xenografts, and biologically active molecules of both protein and non-protein nature), synthetic materials (for example, polyethylene glycol and polylactide), which are obtained by chemical synthesis, composite materials which combine several biological and/or synthetic materials.

TRANSPLANTS

When discussing cartilage transplants, it is essential first to classify them into three categories: autologous transplants (the donor is the same as the recipient), allogeneic transplants (the donor and recipient belong to the same species), and xenotransplants (the donor and recipient belong to different species).

Autotransplantation. Autologous repair is considered the "gold standard" in regenerative medicine. Since the graft is harvested directly from the recipient's own donor site, many immunological issues associated with defect repair are eliminated. In practice, small fragments of cartilage tissue or pieces of bone tissue with overlying cartilage are often used as grafts [2].

The primary drawback of this approach is the extremely limited volume of tissue that can be harvested. Apart from that, extra surgical interventions are required for the autopsy of the graft, which may cause pain at the donor site [24]. Nevertheless, according to I.M. Zazirny and R.Ya. Shmigelski (2015), more than 70% of the interventions resulted in improved outcomes [3]. In cases of massive cartilage surface defects, the insufficiency of donor tissue and the limited available harvest sites become a significant problem. To overcome this issue, some groups of clinicians have adopted a combined repair approach that uses autotransplantation supplemented by various materials (including collagen sponges) to compensate for the lost volume of articular cartilage [5].

Allotransplantation. Another approach to address the challenges associated with autotransplantation is the use of allogeneic transplants. Cartilage allografts were actively used until around 2010, after which the number of publications on the subject began to decline. Nevertheless, this method has been studied extensively. Typically, the grafts consist of fragments of bone tissue with an adjacent layer of cartilage [51], which is due to the fact that cartilage receives nourishment not only from the synovial fluid but also via diffusion from the subchondral bone [49].

Since 1981, allotransplantation has been introduced into pediatric orthopedic practice by Professor V.L. Andrianov, who proposed to use a demineralized bone-cartilage allograft (DBCA) of cadaveric origin for treating the consequences of acute hematogenous osteomyelitis of the proximal femur, which was accompanied by destructive hip dislocation. Later, in 1992, S.V. Filatov proposed the use of perforated DBCA, and the technique was validated by satisfactory functional outcomes in the postoperative period. The surgical technique involves reshaping the femoral head into a spherical form in cases of pronounced deformity and fixing the graft with its cancellous surface facing the acetabulum, followed by joint decompression.

The cadaveric origin of the graft can significantly increase the available donor material compared to autografts. It is worth mentioning that cadaveric grafts have been widely used in the production of composite materials [14].

Allogeneic grafts require specific preparation and preservation methods for transportation. Currently, there is no consensus on which preservation method for cartilage tissue is preferable for future transplantation into the defect area. The main approaches which minimally affect the structure of cartilage tissue are divided into two types: the use of native chondral structures and the application of cryogenic technologies to preserve the cartilage for later implantation [10].

Xenotransplants. In many countries, ethical and legal challenges complicate the preparation of allografts. At the same time, the availability of animal tissues makes xenotransplants an attractive alternative to allo- and autotransplantation.

Xenogeneic transplants are tissues obtained from various animals, particularly pigs and cattle. In many cases, it is not the tissue fragments themselves that are used, but rather cells harvested from the animal donor [6].

The primary challenge associated with xenotransplants is their immunogenicity. Various approaches have been employed to reduce it, including lyophilization, freezing, chemical treatment, and gamma irradiation. However, due to the unique composition of cartilage tissue, these methods can lead to a reduction in its chondrogenic potential. Another significant concern is the potential transmission of infections [1].

Despite these challenges, the literature reports both positive and negative outcomes in experimental studies [63]. In a study [80], it was suggested that the observed results might be related to the duration of the studies. In short-term experiments, the outcomes were better than in long-term ones. Moreover, the choice of the experimental model and the corresponding type of recipient plays a crucial role: studies conducted in small rodents have obtained better results than those in other species.

BIOLOGICAL POLYMERS

Natural polymers such as collagen, chitosan, alginate, gelatin, and many others are actively employed in cartilage tissue engineering. Many of these natural polymers exhibit high cell affinity, are easily modified, resorbed, and effectively mimic the extracellular matrix (ECM) of cartilage tissue. However, their autonomous use is limited by low mechanical properties and, in many cases, a high resorption rate, which does not allow the effective restoration of cartilage function.

Collagen. Collagens are a family of proteins that are among the most widely represented in the human body.

They constitute the most important component of the ECM and, in their native state, offer excellent biocompatibility, low immunogenicity, and bioresorbability. Collagens are composed of polypeptide chains that contain tripeptide sequences of glycine, proline, and hydroxyproline. These tripeptide sequences form a structure that ensures the stability and mechanical properties of collagen matrices [76].

Collagens serve as an excellent matrix for cultivating various cell lines and actively interact with growth and differentiation factors, thereby enhancing the proliferation and adhesion of cell cultures [74]. Collagen matrices can be produced from collagen obtained from fish [79], cattle [86], or recombinant human collagen [88]. Despite their outstanding biological characteristics, collagens exhibit low mechanical strength [35] and a high rate of biological resorption [31], which considerably limits their application — especially for articular cartilage replacement.

A primary strategy to overcome the limitations of collagen is to employ composite materials. For example, in study [29], polylactide and chitosan were used to improve the mechanical properties of collagen matrices. Other studies have modified mechanical characteristics by incorporating elastin, polyglycolic acid (PGA), or polyethylene glycol (PEG) [61].

While collagen itself is an excellent biological polymer for cell cultivation and implantation into defect areas (as demonstrated in [19]), the addition of various biologically active molecules can further enhance tissue repair processes or influence cell proliferation on collagen matrices [68]. Since chondrogenesis is closely linked with osteogenesis, bone morphogenetic proteins (BMPs) are often used [69] — under certain conditions, they can direct the differentiation of mesenchymal stem cells (MSCs) toward a chondrocytic way and affect the rate of ECM formation during long-term *in vitro* cultivation on collagen substrates [46].

Chitosan. Chitosan is a natural, hydrophilic, polycationic biopolymer obtained from chitin. Structurally, it resembles cartilage and bone tissue, which makes it a good candidate for mimicking the ECM [78].

Chitosan is a deacetylated product of chitin and is composed of β-(1→4)-2-acetamido-D-glucose and β-(1→4)-2-amino-D-glucose units [18]. Due to the presence of amino and hydroxyl groups, the polymer forms both intermolecular and intramolecular hydrogen bonds. The abundance of multifunctional surface chemical groups enables modification of its surface with growth factors and cell differentiation factors [8]. Chitosan exhibits excellent biological and cytological compatibility and bioresorbability; its surface easily facilitates the formation of a protein coating, creating a native-like environment for cells [23].

The disadvantages of chitosan include low mechanical strength and poor thermal stability. These issues are typically handled by applying composite materials; for example, a study [41] utilized polylactide. PEG has also been applied to improve its mechanical properties [89].

Alginate. Alginate is a polysaccharide obtained from brown algae. It is widely used in medicine due to its biocompatibility and non-immunogenicity. Due to its gel-like structure, alginate serves as an excellent substrate for cell growth [21].

Alginate is composed of two types of blocks: D-mannuronic acid (M-block) and L-guluronic acid (G-block). Variations in the ratio and chain lengths of these blocks lead to changes in the mechanical characteristics of alginate scaffolds [12].

Alginate is degraded by enzymes of the alginate lyase class which are not typically found in mammals rendering this material essentially non-resorbable when implanted *in vivo*. Nonetheless, it exhibits a high capacity for chondrogenesis and osteogenesis, permitting its use in both *in vitro* and *in vivo* studies [48]. Another limitation of alginate is its gelation, which prevents the formation of complex porous structures.

To handle these issues, various composite formulations have been developed, they combine alginate with chitosan [67], collagen [32], or numerous synthetic polymers [75] to impart additional mechanical and biological properties. Like many other biological polymers, alginate is frequently used in conjunction with growth and differentiation factors [25]. Moreover, numerous studies have demonstrated the positive effect of incorporating hydroxyapatite particles into alginate matrices [92].

Silk fibroin. Silk fibroin is one of the oldest biomedical polymers. It consists of thin fibroin fibers coated with a globular protein known as sericin. The presence of this foreign protein often triggers an immune response; as a consequence, several simple and accessible methods — physical, enzymatic, and chemical — have been developed to remove sericin from fibroin fibers [47]. Silk fibroin is taken from various organisms and is subsequently purified to remove sericin. Depending on the source and processing methods, the mechanical properties of fibroin fibers can vary [64].

Due to their fibrous structure, materials derived from fibroin can tolerate prolonged cyclic loading — an important feature for implantation as a replacement for defective cartilage tissue [37]. Besides that, fibroin-based constructs exhibit a long *in vivo* resorption time, allowing for the gradual replacement of cartilage tissue [38].

The primary disadvantage of silk fibroin is its immunogenicity; despite high-quality purification processes, there

is considerable evidence of delayed immune responses to both silk fibers and the implanted constructs [26].

Hyaluronic Acid. Hyaluronic acid is a disaccharide composed of N-acetylglucosamine and glucuronic acid. It is favored because it is one of the main components of synovial fluid, naturally supports chondrocyte proliferation, and enhances cartilage tissue repair. Its molecular structure promotes easy cell adhesion to its surface [52].

Hyaluronic acid is a resorbable, biocompatible, and non-toxic material. Depending on its molecular weight, it exhibits varying mechanical properties and lubricating characteristics — both essential for cartilage tissue function [96]. According to [36], at certain shear rates, hyaluronic acid behaves similarly to water, which limits its use as a friction-reducing material on joint surfaces.

With advances in bioprinting using hydrogels, hyaluronic acid is now used either as a base material or as a coating for various printed constructs [84]. In addition, hyaluronic acid is used in composite formulations with alginate [11], collagen, and gelatin [58] as bio-inks for 3D bioprinting. Its widespread use as a component in intra-articular injections for gonarthrosis of varying severity has long been established as an effective and minimally invasive method [95].

Gelatin. Gelatin is a fibrous protein obtained from partially hydrolyzed collagen. It exhibits high biocompatibility and is bioresorbable, which makes it suitable for various medical applications. Due to its functionalization, gelatin is widely used for drug delivery and in tissue engineering. Its polyionic nature allows for the easy conjugation of polysaccharides, growth and differentiation factors, proteins, nucleotides, and other therapeutic molecules [59].

In recent years, gelatin has become important in the development of materials for cartilage tissue engineering due to the ease with which printed samples can be stabilized post-3D printing. In particular, methacryloyl gelatin (GelMA) has attracted considerable attention. Hydrogels based on GelMA possess an ECM-like structure, enabling the creation of scaffolds that closely mimic native tissue [91]. GelMA can be synthesized by various methods, which allows for the modulation of the mechanical and chemical characteristics of the resulting matrices [43]. However, according to a study [85], methacryloyl gelatin may have negative effects on cell cultures, which is attributed to the need for photoinitiators during the crosslinking process following printing.

Bacterial cellulose. Among naturally occurring polymers, cellulose is one of the most common. It forms the cell walls of plants and is also secreted by many bacteria [7]. Bacterial cellulose (BC) is preferred because it has a more branched nanofiber structure, providing a greater surface area at the same volume. According to [70], cellulose fibers are easily modified, which allows one to modify the structure and properties of matrices based on BC.

The mechanical strength, crystallinity, and moisture-retention characteristics of bacterial cellulose depend not only on the type of bacteria used to produce the material but also on the composition of the culture medium, the addition of various substances, and the cultivation conditions. Despite these advantages, BC exhibits a very long resorption period, and cells do not show a high degree of adhesion to its surface [66]. The main method of dealing with cell compatibility issues is the addition of collagen [94] or alginate [65].

Synthetic polymers

Synthetic polymers have a longer resorption period compared to natural polymers, and controlling the degree of polymerization makes it possible to influence mechanical characteristics, matrix structure, and degradation.

Synthetic polymers are generally preferred because of their superior mechanical properties compared to natural polymers. Nevertheless, purely synthetic polymers are currently almost never used independently for cartilage tissue repair due to low cell compatibility and the absence of therapeutic features. In most cases, synthetic polymers — such as polyglycolic acid, polylactic acid, polyethylene glycol, and polycaprolactone — serve as scaffolds in combination with natural polymers, cells, and agents that enhance proliferation and influence cell differentiation.

PGA. Polyglycolic acid (PGA) is a linear crystalline hydrophilic polyester. This polymer demonstrates good adhesive properties, it is non-toxic and bioresorbable, and has high hygroscopicity, which allows it to be used as a cell carrier in cartilage tissue repair [13].

Due to the specifics of cartilage tissue regeneration, polyglycolic scaffolds are often used together with cell cultures [93]. Various substances that influence tissue differentiation at the implantation site are also widely applied [30].

Like other polyesters, PGA is responsive to extrusion, injection molding, and compression molding [73]. Some studies [27, 33] have shown that PGA can serve as an independent material for 3D printing. In addition, many research workers employ the copolymerization of PGA with polylactide (PLA) to obtain the copolymer PLGA [20], which makes it possible to control printing quality as well as the hydrophilic properties of the material. It should also be noted that PGA degradation leads to the release of acidic products, which reduces the material's biocompatibility and can cause inflammatory reactions at the implantation site. A partial solution to this issue is the use of compositions with polylactide [40].

PLA. Polylactide is a linear polyester with lower crystallinity than PGA. Its key advantages include thermal stability, biocompatibility, and the non-toxicity of both the material itself and its resorption products. Polylactide has high viscosity and thermoplasticity; therefore, it is primarily used for 3D printing and for manufacturing scaffolds for tissue repair [22].

According to [54, 87], polylactide matrices can be used as autonomous cell carriers; however, the addition of biological polymers improves their *in vitro* compatibility by enhancing cell adhesion and proliferation [45]. Growth factors are also employed for the same purposes, as in the case of PGA [90].

PEG. Polyethylene is a water-soluble polymer that is not recognized by the immune system [17]. Two main markings are used for polyethylene: polyethylene glycol (PEG) with a molecular weight below 20,000 Da, and polyethylene oxide (PEO) with a higher molecular weight.

Due to its solubility, interest in polyethylene has grown in recent years. Polyethylene is increasingly used in 3D printing as a carrier gel [42]. Nonetheless, its native mechanical characteristics are inadequate for it to serve as an autonomous material in tissue engineering. For that reason, numerous composite materials with various synthetic polymers have been proposed [28].

The primary advantage of this polymer is its rapid and almost unhindered elimination from the body. By binding to other substances, including resorption products, polyethylene can also intensify their excretion [16]. Thanks to this property, polyethylene glycol is frequently employed as a carrier for drug delivery [53], including the delivery of growth factors to the implantation site [82].

PCL. Polycaprolactone is a synthetic semicrystalline polyester characterized by high mechanical strength, elasticity, and biocompatibility, and it is also bioresorbable [77]. Like PEG, its degradation products are easily excreted from the body [55]. Polycaprolactone is widely used in cartilage surgery due to its biomechanical properties, which closely resemble those of native tissue [81].

However, polycaprolactone is hydrophobic, which is its main disadvantage since cells cannot easily spread on its surface, leading to poor adhesion and, consequently, low viability of cell cultures [83]. For this reason, this polymer is primarily combined with other substances (for example, polylactide) to improve its mechanical properties [72]. Another approach to enhancing cell adhesion is to add natural polymers, to which cells actually adhere, while the polycaprolactone serves as a supporting scaffold [39]. Numerous studies use various agents to improve cell adhesion, particularly hydroxyapatite particles, which coat the surface and enable cells to attach more effectively [57].

CONCLUSION

Effective restoration of cartilage tissue damage remains a challenging, yet highly significant task. As demonstrated in this article, the most frequently used approaches and materials have numerous disadvantages. The independent use of natural biological polymers enables to create constructs exhibiting biocompatibility and affinity for cell cultures; however, these materials possess very low mechanical properties. This issue can be handled by employing synthetic polymers, which, in turn, have a longer resorption period, can tolerate prolonged static and dynamic mechanical loads, and may be used for cartilage tissue repair. At the same time, the autonomous use of synthetic polymers is limited by poor adhesion of cell cultures to their surfaces.

As indicated by multiple sources, the integration, proliferation, and regeneration of cartilage tissue at the implantation site can be accelerated by using various additional agents, especially growth and differentiation factors. Composite constructs pre-loaded with cell cultures and various factors on their surfaces demonstrate better outcomes compared to implantation of composite or single materials.

The development of constructs for bone tissue engineering requires the use of various synthetic and natural polymers to ensure that the designed constructs mimic the biological and mechanical characteristics of native cartilage tissue. It is also necessary to apply multiple biologically active molecules and cell cultures, thereby allowing the construct to approximate native tissue as closely as possible and speeding recovery in the postoperative period.

ADDITIONAL INFORMATION

Author contribution. Thereby, all authors made a substantial contribution to the conception of the study, acquisition, analysis, interpretation of data for the work, drafting and revising the article, final approval of the version to be published and agree to be accountable for all aspects of the study.

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MESENCHYMAL STEM CELLS MIGRATION MECHANISMS AND POSSIBLE STRATEGIES FOR THEIR IMPROVEMENT

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Abstract. In recent decades a lot of data have been accumulated on the mechanisms of tissue renewal and regeneration, which would be impossible without the participation of stem cells. It has been proven that these processes in many tissues are carried out by tissue-specific stem cells (TSCs), but their production, cultivation and administration for therapeutic purposes are extremely difficult. Along with this, mesenchymal stem cells (MSCs) are a promising therapeutic agent that has already proven its clinical effectiveness in various diseases and in tissue engineering. One of the features of MSCs introduced systemically is the ability to find a niche in the affected tissue and remain there, having a significant impact on inflammation, tissue remodeling processes and its regenerative potential. However, the mechanisms of differentiation and migration of MSCs, as well as the factors influencing these processes, are not fully disclosed. This review makes an attempt to summarize the accumulated data on the mechanisms of MSC migration and possible ways to improve it.

Key words: mesenchymal stem cells; tissue regeneration; cell therapy; migration.

МЕХАНИЗМЫ МИГРАЦИИ МЕЗЕНХИМАЛЬНЫХ СТВОЛОВЫХ КЛЕТОК И ВОЗМОЖНЫЕ СТРАТЕГИИ ИХ УЛУЧШЕНИЯ

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Резюме. В последние десятилетия накапливается все больше данных о механизмах обновления и регенерации тканей, которые были бы невозможны без участия стволовых клеток. Доказано, что данные процессы во многих тканях осуществляются за счет тканеспецифичных стволовых клеток (ТСК), однако их получение, культивация и введение с терапевтической целью крайне затруднительны. Наряду с этим наибольший интерес

представляют мезенхимальные стволовые клетки (МСК), которые, благодаря возможности их выделения, экспансии и мультипотентности, являются многообещающим терапевтическим агентом, уже доказавшим свою клиническую эффективность при различных нозологиях, в том числе в вопросах тканевой инженерии. Одной из особенностей МСК, введенных системно, является способность находить нишу в пораженной ткани и оставаться в ней, оказывая существенное влияние на воспаление, процессы ремоделирования ткани и ее регенеративный потенциал. Однако механизмы дифференцировки и миграции МСК, а также факторы, влияющие на эти процессы, раскрыты не полностью. В данном обзоре обобщены современные данные о механизмах миграции МСК и возможных путях ее улучшения.

Ключевые слова: мезенхимальные стволовые клетки; регенерация тканей; клеточная терапия; миграция.

The assumption that there are cells in the body that promote wound healing was made by Cohnheim back at the end of the XIX century [13]. Mesenchymal stem cells (MSCs) were first isolated and cultured in 1968 by Friedenstein, who discovered that transplantation of cell colonies into semi-syngeneic animals could lead to the formation of cartilage and bone tissue containing bone marrow [17]. Years later, it was realized that these works described cells with multipotent ability. Further studies of the heterogeneous population of bone marrow MSCs were carried on by a group of scientists led by Kaplan in the 1980–1990. During this period, the possibility of differentiation of MSCs into various mesenchymal tissues was discovered for the first time, and the first surface markers characteristic of MSCs (CD73, CD105) were identified [21]. The term "mesenchymal stem cells" itself was proposed in 1991 [12]. Since then, the era of cell therapy began.

According to the accumulated data, MSCs show a good safety profile, have multilineage differentiation potential and a low immunogenic profile, which makes them an attractive therapeutic agent [20]. By 2018, estimates of the number of patients who have had experience with the therapeutic use of MSCs ranges from 10,000 to 70,000 people, including children [11]. No serious adverse events associated with MSC therapy and requiring early termination of the clinical trial were reported [11].

FEATURES OF MSC PHENOTYPE

MSCs were initially characterized by their ability to generate colony-forming units, fibroblasts (CFU-Fs). The number of CFU-Fs in the bone marrow is about one cell per 10^4–10^5 mononuclear cells [16]. MSCs are characterized by the expression of various surface markers, but none of them appear to be expressed exclusively by MSCs. In this regard, the International Society for Cell and Gene Therapy (ISCT) proposes at least three conditions that can characterize MSCs [52]:

- adhesion to specialized plastic under standard cultivation conditions;
- expression of surface markers CD105, CD73 and CD90; in this case, CD11b, CD14, CD19, CD34, CD45, CD79α and HLA-DR, which are markers of hematopoietic stem cells, should not be present;
- ability to differentiate into osteoblasts, adipocytes and chondroblasts *in vitro*.

However, controversy remains regarding the ideal set of MSC surface markers, since many of them are expressed by other cell types and may also vary depending on the source, MSC culture method, and number of passages in culture media. For example, a number of surface markers (Oct-4, Nanog, Rex-1, SSEA-3, etc.) are expressed on MSCs, isolated from peripheral blood, liver and bone marrow of a fetus in the first trimester of pregnancy, but are absent on MSCs isolated from the bone marrow of adults [41].

According to the data obtained using multichromatic flow cytometry, MSCs change their immunophenotypic profile depending on the passage number (1–8), although the expression of some markers is variable and independent of time [36]. In particular, during the first passages, high expression of CD29, CD166 and CD201 is observed in addition to the canonical markers CD73, CD90 and CD105. At the same time, by the 8th passage, differences are observed in the expression of CD34, CD200 and CD271 by MSCs, which requires further study, especially in terms of clinical use.

The ability to express surface markers (CD13, CD29, CD44, CD73, CD90, CD105, CD146, CD166) significantly decreases after the 7th passage and beyond, and MSCs themselves enter the aging phase and lose the ability to proliferate [55]. In this regard, for therapeutic purposes it is preferable to use MSCs that have undergone less than 6 passages *in vitro* [1].

STAGES OF MSC MIGRATION TO DAMAGED TISSUE

The therapeutic efficacy of MSCs largely depends on their ability to produce juxtacrine and paracrine factors. For juxta- and paracrine effects to be possible, migration of MSCs into the affected organ/tissue is necessary, which may depend on many factors, including the age of the donor, the number of passages of MSCs, the conditions of their cultivation and the method of delivery to the target organ [3, 4].

It has been shown that when administered systemically, MSCs undergo a multi-stage process of transition from the

bloodstream to the target tissue. Systemic recruitment of MSCs can be divided into five stages: 1) attachment to the endothelial surface; 2) activation; 3) arrest; 4) diapedesis and 5) migration to the target. The initial binding of MSCs to endothelial cells is facilitated by the expression of selectins. MSCs express CD44, which was first identified as a lymphocyte receptor responsible for homing. CD44 interacts with selectins and promotes the process of "rolling" MSCs along the vascular wall [43]. To demonstrate the binding of MSCs to endothelial cells, a parallel plate flow chamber seeded with endothelial cells was created [42]. Antibodies to P-selectin were shown to inhibit the binding of MSCs to endothelial cells, whereas immobilization of P-selectin resulted in rapid binding of MSCs to endothelial cells. As MSCs do not express PSGL-1, it is assumed that they must use another ligand for this purpose. Galectin-1 has been identified as one of these ligands [49]. Another study identified CD24 as a potential P-selectin ligand for MSCs isolated from adipose tissue [7].

The second step (activation) is mediated by G protein-coupled chemokine receptors, usually in response to proinflammatory signals. Expression of stromal cell-derived factor-1 (SDF-1), a ligand for the chemokine receptor CXCR4, is crucial for this stage [30]. Expression of SDF-1 on MSCs has a direct impact on the rate of their migration to the site of damage in a rat model of myocardial infarction [61]. MSCs have also been shown to express CXCR7, which similarly binds to SDF-1 to facilitate homing to various tissues [31]. Overexpression of CXCR4 on MSCs promotes their return to the bone marrow [10]. Along with CXCR4, expression of the chemokine CCL2 on cardiomyocytes of transgenic mice with induced myocardial ischemia is able to enhance the migration of MSCs expressing the corresponding CCR2 receptor due to direct interaction between the ligand and the receptor [8]. A number of studies have shown that MSCs, both freshly isolated and at the cultivation stage, are capable of expressing CCR1, CCR4, CCR7, CCR10, CCR9, CXCR5 and CXCR6 [22, 53], but their role remains to be clarified.

The third stage (arrest) is mediated by integrins. MSCs can express the integrin receptor VLA-4, consisting of α4 (CD49d) and β1 (CD29) chains, which is activated in response to chemokines such as SDF-1. Once activated, VLA-4 binds to VCAM-1 on endothelial cells [47]. It has been shown that neutralizing antibodies to the β1 chain of VLA-4 inhibit the homing of MSCs to the ischemic myocardium, which cannot be said about antibodies blocking the α4 chain [24]. Overexpression of the VLA-4 α4 chain is thought to promote MSC return to the bone marrow [29]. An interesting fact is that MSCs, along with endothelial cells, are capable of expressing cell adhesion molecules VCAM-1 (ligand for VLA-4), as well as ICAM-1 (ligand for the integrin receptor LFA-1) [28].

At the next (fourth) stage, MSCs must pass through the endothelial cell layer and the basement membrane (transmigration) into the extravascular space. To achieve this, MSCs secrete matrix metalloproteinases (MMPs) [47]. A similar mechanism is used by white blood cells and tumor cells for a similar purpose. The expression of MMPs is determined by the secretion of proinflammatory cytokines, which serve as a signal for cell migration into damaged tissue. The maturation and activity of MMPs are regulated by various proteins, most notably tissue inhibitors of metalloproteinases (TIMPs). It is assumed that the balance of MMPs/TIMPs influences the rate of migration of MSCs through the endothelium. When neutralizing antibodies to MMP-2 (an enzyme possessing ability to break down the main component of the basement membrane (collagen IV)) are added to the culture medium, it leads to a significant decrease in MSC migration *in vitro*. A similar result is observed when TIMP3 is added to the culture medium [14]. Neutralization of TIMP1 enhances the migration of MSCs through the endothelium, while neutralization of MMP2, MT1-MMP or TIMP2 reduces it [40]. The question of the participation of various MMPs and TIMPs in MSC migration requires further study.

In the fifth stage, MSCs must migrate to the site of injury, usually in response to signals released from the damaged tissue, such as basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), insulin-like growth factor-1 (IGF-1), platelet-derived growth factor (PDGF) and transforming growth factor β1 (TGF-β1).

Platelet-derived growth factor-AB (PDGF-AB) and insulin-like growth factor-1 (IGF-1) influence MSC migration to a greater extent than the RANTES chemokines, macrophage chemokines (MDC) and stromal-derived factor-1 (SDF-1), which have a limited effect [38]. Preincubation of MSCs with the tumor necrosis factor TNFα increases their migration towards chemokines, probably due to activation of the CCR2, CCR3 and CCR4 receptors. Proinflammatory interleukin-8 (IL-8) can promote the migration of MSCs to the site of injury, as well as their secretion of vascular endothelial growth factor (VEGF), which was shown in a rat model of a stroke [9]. Administration of MSCs treated with IL-8 leads to a decrease in the volume of brain damage and increased angiogenesis in the ischemic border zone compared to MSC therapy without IL-8.

The bFGF factor, being a powerful mitogen, can stimulate the migration of various types of cells, in particular MSCs [33]. A low concentration of bFGF promotes MSC migration, while a high concentration of bFGF inhibits MSC migration, and this contradictory effect of bFGF allows for their directional routing [45]. One of the possible mechanisms for enhancing MSC migration is supposed to be their enhanced expression of αVβ3 integrin and activation of the MEK/ERK signaling pathway. In addition to recruitment, bFGF promotes increased secretion of VEGF by MSCs, which is important in restoring vascular integrity after damage of endothelium [50].

IGF-1, which is actively involved in the regulation of the processes of growth and differentiation of various cells of the body, can also influence the migration of MSCs. Overexpression of IGF-1 on MSCs improves survival and transplant engraftment in a rat model of infarction and promotes MSC recruitment, likely through the paracrine release of SDF-1 [23]. Pre-incubation of MSCs with the addition of IGF-1 to the culture medium improves the migration ability of MSCs in a model of acute kidney injury, with the presence of MSCs promoting rapid normalization of kidney function [57]. IGF-1 increases the migratory potential of MSCs by increasing the expression of the chemokine receptor CXCR4 and its ligand SDF-1. The response to SDF-1 can be attenuated by a PI3 kinase inhibitor, but not by an inhibitor of mitogen-activated protein/ERK kinase, which shows the importance of the PI3/Akt pathway in the response of MSCs to various signaling molecules [32].

TGF-β1 has a broad biological activity, playing an important role in cell growth, differentiation and immune regulation of cells. Remaining in an inactive form in the cell matrix, TGF-β1 is released in an active form in response to mechanical stress or inflammation and is involved in the repair and regeneration of damaged tissues. The expression of TGF-β1 increases during ischemia/reperfusion injury of the myocardium of mice, which enhances the recruitment of MSCs by regulating the expression of CXCR4 [60]. In a mouse model of asthma, it was shown that high levels of active TGF-β1 in their lung tissue were associated with allergen stimulation, and increased migration of MSCs into the lungs was observed. It has also been shown that intraperitoneal administration of both TGF-β1-neutralizing antibodies and a TβR inhibitor to experimental animals leads to a decrease in the migratory ability of MSCs [19].

From the above it follows that chemical factors influencing MSC migration act in a complex manner, activating different signaling pathways. Understanding the molecular events that promote MSC migration has significant implications for strategies to optimize their delivery for therapeutic purposes.

STRATEGIES TO IMPROVE MSC DELIVERY TO TARGET TISSUE

Despite large doses of MSCs when administered systemically (≈1 million MSCs per 1 kg of patient body weight), only a small part of them actually reaches the target tissue [15]. This is believed to be due to several factors. After systemic administration a significant part of MSCs are retained in the capillaries of the lungs [44]. The therapy received by the patient may influence the migratory ability of MSCs. Vasodilators and anticoagulants such as heparin have been shown to reduce the uptake of MSCs into the lungs and increase the number of MSCs in other organs, particularly the liver and red bone marrow [18]. However, the migration process of MSCs is determined, as described above, by the expression profile of specific surface molecules and their receptors, and not simply by passive spread through the vasculature. Another problem is that on MSC after expansion *in vitro*, the expression of molecules required for migration to the target tissue appears to be reduced [22]. There is also heterogeneous expression of homing molecules in MSC cultures from different sources, such as those isolated from adipose tissue versus those isolated from bone marrow [48].

All of these factors necessitate the development of strategies that improve the delivery of MSCs to the target tissue. The most discussed approaches are: *introduction of MSCs into the target tissue, magnetic targeting, pre-treatment of MSCs in the culture or changing the culture conditions, merging the MSC culture with other cell cultures.*

Introduction of MSCs into the target tissue or nearby locations is the simplest and most intuitive strategy to increase the presence of MSCs in the lesion. Unfortunately, there are few studies comparing the effect of different methods of MSC delivery on the results of the therapy; however, there is convincing evidence of some advantages of non-systemic administration compared to systemic administration. It has been shown that transcatheter administration of MSCs in patients with ischemic cardiomyopathy after myocardial infarction increases myocardial contractility in the area of the permanent scar, which influences the subsequent reverse tissue remodeling. However, the study design did not provide for systemic administration [56]. According to the meta-analysis carried out by Vu, in ischemic stroke, intracerebral administration of MSCs appears to lead to a significant improvement in neurological status when compared with intra-arterial and intravenous introduction of MSC [54]. In a porcine model of myocardial infarction, it was shown that transendocardial administration of MSCs reduces the infarct area, while intramyocardial, intracoronary and intravenous administration does not lead to significant improvements [26]. However, another meta-analysis reported that MSC administration improved left ventricular ejection fraction in patients after myocardial infarction in case of intracoronary, intravenous, and intramyocardial administration of MSCs in descending order of effect size [25].

In acute lung injury syndrome, intravenous administration is more effective than intraperitoneal administration [35]. However, the method of administration of MSCs does not influence the results of therapy for traumatic brain injuries [37]. Obviously, one should not assume that direct injection of MSCs into the target tissue will provide the best results.

Another approach to targeting MSCs to target tissue is magnetic targeting, in which cells labeled with magnetic particles are guided to the target organ using an external magnetic field. MSCs labeled with iron oxide were administered intravenously

to rats with a magnet attached to the body in the projection of the liver and to rats without a magnet. In rats that wore an external magnet, 15 days after MSC injection, there were approximately 2 times more labeled MSCs in the liver compared to the control group. In rats that did not wear magnets, MSCs were predominantly localized around the portal triads, and in rats that wore magnets, MSCs were recorded deep in the liver parenchyma [6]. Yanai et al. were able to concentrate MSCs labeled with magnetic particles in the projection of the retina in rats, both when injected into the retina and when administered intravenously using a magnet placed in the orbital area. In rats wearing an external magnet, higher levels of anti-inflammatory factors (IL-10; hepatocyte growth factor (HGF)) were noted, which indicates the therapeutic effect of MSCs [58]. Another study used a magnet to concentrate magnetically labeled MSCs into damaged olfactory bulbs. These cells were detected one week after injection and were present in higher numbers compared to MSCs not treated with magnetic particles. It was noted that magnetic iron oxide particles increased the expression of CXCR4 and SDF-1 on MSCs [59].

Due to the fact that the cultivation of MSCs *in vitro* reduces the expression of surface molecules involved in recruitment on them, *pre-treatment of MSCs in culture or changing culture conditions* is considered to be the simplest and most accessible strategy to enhance MSC migration into target tissues. One way to achieve this goal is to add cocktails with cytokines and other growth factors to the culture medium at the stage of MSC expansion. The combination of the cytokine receptor flt3, stem cell factor (SCF), IL-3, IL-6 and hepatocyte growth factor (HGF) increases both intracellular and membrane expression of CXCR4 on cultured MSCs, which enhances their migratory ability towards SDF-1 [46]. CXCR4 expression can also be enhanced by adding glycogen synthase kinase-3β (GSK-3β) inhibitors to MSC culture, resulting in improved migratory ability *in vitro*, without influencing cell viability [27]. Short-term pretreatment of MSC culture with valproic acid leads to an increase in the expression of CXCR4 and MMP-2 on MSCs and increases their migration towards SDF-1, without influencing the ability of MSCs to differentiate [34].

Culture conditions also influence CXCR4 expression on MSCs. It is believed that this depends on the presence of hypoxia-inducible factor 1α (HIF-1α). Cultivation under hypoxic conditions leads to increased expression of CXCR4 and improved migration of MSCs both *in vitro* and *in vivo*, with this effect being observed both during short-term oxygen limitation and in response to prolonged cultivation under hypoxic conditions [5]. It is worth noting that hypoxia can influence the enhancement of adipogenic and osteogenic differentiation of MSCs in culture, which may be undesirable for further therapeutic use [51].

As noted previously, MSCs express low levels of CXCR4, so a number of researchers have attempted transfection or transduction, in which CXCR4 expression plasmids are delivered into the MSC nucleus using viruses. In approximately 90% of cases after treatment of MSCs with a retrovirus (*ex vivo*) there is overexpression of CXCR4, which leads to phosphorylation of AKT mitogen-activated proteins, as well as an increase in the expression of matrix metalloproteinases (MMPs) after SDF-1 stimulation. MSCs demonstrate enhanced migratory ability towards SDF-1 and homing into the bone marrow of NOD/SCID mice [10]. Viral transduction is the most effective method for obtaining high and stable levels of expression in target cells, but it is associated with the risk of oncogenic transformation and is a rather expensive method.

Fusion of *cell cultures* can be considered within the framework of the approach of enhancing the migration of MSCs, while there are isolated reports on this topic. Co-culture of MSCs derived from amniotic fluid with amniotic epithelial cells enhances the proliferation and expression of CXCR4 [39]. Co-culture of MSCs isolated from rat adipose tissue with Sertoli cells enhances the proliferation and migration of MSCs, apparently due to the activation of the MAPK/ERK1/2, MAPK/p-38 and PI3K/Akt signaling pathways. Treatment of MSCs with conditioned media obtained from endothelial cell cultures increases MSC migration *in vitro*, possibly due to the presence of the cytokines IL-6 and IL-8 [2].

Thus, mesenchymal stem cells have the ability, when administered systemically, to enter the affected tissue and influence inflammation, remodeling processes and regeneration, therefore, further clarification of the mechanisms of differentiation and migration of MSCs, identification of factors influencing these processes will contribute to the expansion of their use in many fields of medicine.

ADDITIONAL INFORMATION

Author contribution. Thereby, all authors made a substantial contribution to the conception of the study, acquisition, analysis, interpretation of data for the work, drafting and revising the article, final approval of the version to be published and agree to be accountable for all aspects of the study.

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LOWER EXTREMITY PERIPHERAL ARTERY DISEASE: CONTEMPORARY EPIDEMIOLOGY, MANAGEMENT AND FUTURE TRENDS (A SCIENTIFIC STATEMENT)

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Abstract. Lower extremity peripheral artery disease (PAD) affects >230 million adults worldwide and is associated with increased risk of various adverse clinical outcomes (other cardiovascular diseases such as coronary heart disease and stroke and limb outcomes such as amputation). Despite its prevalence and clinical importance, PAD has been historically underappreciated by health care professionals and patients. This underappreciation seems multifactorial (eg, limited availability of the first-line diagnostic test, the ankle-brachial index, in clinics; incorrect perceptions that a leg vascular disease is not fatal and that the diagnosis of PAD would not necessarily change clinical practice). In the past several years, a body of evidence has indicated that these perceptions are incorrect. Several studies have consistently demonstrated that many patients with PAD are not receiving evidence-based therapies. Thus, this scientific statement provides an update for health care professionals regarding contemporary epidemiology (eg, prevalence, temporal trends, risk factors, and complications) of PAD, the present status of diagnosis (physiological tests and imaging modalities), and the major gaps in the management of PAD (eg, medications, exercise therapy, and revascularization). The statement also lists key gaps in research, clinical practice, and implementation related to PAD. Mastermind efforts among different parties (eg, health care providers, researchers, expert organizations, and health care organizations) will be needed to increase the awareness and understanding of PAD and improve the diagnostic approaches, management, and prognosis of PAD.

Key words: atherosclerosis; artery; lower extremity; epidemiology; vessel.

ЗАБОЛЕВАНИЕ ПЕРИФЕРИЧЕСКИХ АРТЕРИЙ НИЖНИХ КОНЕЧНОСТЕЙ: СОВРЕМЕННАЯ ЭПИДЕМИОЛОГИЯ, РУКОВОДСТВО И ПЕРСПЕКТИВНЫЕ НАПРАВЛЕНИЯ (НАУЧНОЕ СОЧИНЕНИЕ)

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Резюме. Заболевание периферических артерий нижних конечностей (ЗПА) поражает более 230 миллионов взрослых пациентов во всем мире ежегодно. Это число указывает на высокую распространенность данного заболевания и связанных с ним негативных клинических исходов, например, ишемической болезни сердца, инсульта и ампутации. Исторически ЗПА недооценивалось медицинскими работниками и пациентами по нескольким причинам. Одна из них — ограниченная доступность в клиниках диагностического теста первой линии для ЗПА, такого как лодыжечно-плечевой индекс. Этот тест позволяет определить наличие стеноза и окклюзии артерий нижних конечностей. Еще одна причина — неправильное представление о том, что заболевания сосудов ног не являются фатальными. Однако за последние годы было проведено несколько исследований, которые показали недостаточное использование научно обоснованной терапии у пациентов с ЗПА. Это указывает на то, что нам необходимо обновить свои знания о ЗПА и улучшить практику лечения. Данное исследование представляет собой информацию о современной эпидемиологии ЗПА, включая распространенность заболевания, временные тенденции, факторы риска и осложнения. Оно также освещает современные методы диагностики, такие как физиологические тесты и методы визуализации, и основные пробелы в лечении ЗПА, включая медикаментозную терапию, лечебную физкультуру и реваскуляризацию. Этот научный материал поможет специалистам здравоохранения быть в курсе последних достижений в области ЗПА и обеспечить пациентам оптимальное лечение. В целом болезнь периферических артерий нижних конечностей является серьезной и распространенной проблемой, которая требует внимания и последовательного подхода к диагностике и лечению. Научные исследования играют важную роль в обеспечении оптимальной заботы о пациентах с ЗПА и снижении их риска осложнений.

Ключевые слова: атеросклероз; артерия; нижняя конечность; эпидемиология; сосуд.

DEFINITION OF PAD

Historically, the terms peripheral artery (or arterial) disease and peripheral vascular disease have been used loosely. These terminologies have often included any or all atherosclerotic disease separate from cardiac disease, including carotid artery, renal artery, leg artery, and aortic diseases. Peripheral vascular disease may additionally include peripheral venous and lymphatic disease. In an era of precision medicine, we believe that precise definitions should be used. For the purpose of this scientific statement, we define peripheral artery disease (PAD) as “lower extremity PAD”. Specifically, we are referring to atherosclerotic obstruction from the aortoiliac segments to the pedal arteries.

PREVALENCE AND TEMPORAL TRENDS

Overall PAD

PAD is the third leading cause of atherosclerotic morbidity, following coronary heart disease and stroke. A systematic review of 34 studies (22 from high-income countries and 12 from low- and middle-income countries) demonstrated that the prevalence of PAD was ≈5% at 40 to 44 years of age and ≈12% at 70 to 74 years of age in both men and women in high-income countries. The prevalence of PAD in women in low- and middle-income countries was very similar to that in high-income countries, but the corresponding estimates for men in low- and middle-income countries compared with high-income countries were ≈2% and ≈8%, respectively. Between the years 2000 and 2010, the number of persons living with PAD increased by 13.1% in high-income countries and 28.7% in low- and middle-income countries.

Another recent systematic review estimated that 238 million people were living with PAD in 2015: 64 million living in high-income countries and 172 million living in low- and middle-income countries. Thus, PAD should be recognized as an increasingly global problem. A recent publication from the Global Burden of Disease study also indicates that PAD cases have risen each year since 1990. Similarly, disability-adjusted life-years, years of life lost, and years lived with disability increased over this period. These changes represent population growth rather than a change in age-specific incidence. Worldwide, the age-specific prevalence has been largely steady.

Critical limb ischemia/amputation

Critical limb ischemia (CLI) (or chronic limb-threatening ischemia) is a severe form of PAD and usually defined as PAD with rest pain, nonhealing wounds, or tissue loss [1, 18]. A systematic review has reported that the 1-year cumulative incidence for each of mortality and amputation is ≈20% among patients with CLI [18]. Because few population-based studies have investigated CLI, the epidemiology of CLI is not well understood. Using data from the Market scan database, which includes medical records from large employers' health plans, Medicare, and Medicaid, Nehler et al reported that the prevalence of CLI is 1.3%, accounting for 11% of diagnosed PAD cases, among the eligible study population ≥40 years of age. The rate of CLI admission was constant between 2003 and 2011, with ≈150 per 100 000 population [5].

The rate of lower extremity amputation declined from 2000 to 2009, but since has started to increase in people with diabetes. Specifically, the total annual amputation rate per 1000 individuals with diabetes was 3 in 2009 but exceeded 4.5 in 2015. Although the exact reasons behind this increase in lower extremity amputation in diabetes are unclear, it is important to note that the increase was consistently observed in both major (above ankle) and minor (below ankle) amputations. In the same period, the annual amputation rate in people without diabetes was constant at ≈0.17 per 1000 individuals.

Lifetime risk

Lifetime risk estimate is a useful parameter to communicate long-term risk, especially among younger adults whose 10-year risk estimate is low and thus cannot inform long-term decision-making of preventive therapies. The American Heart Association (AHA) and the American College of Cardiology (ACC) 2018 Guideline on the Management of Blood Cholesterol provides a lifetime risk algorithm for people 20 to 59 years of age but does not take into account PAD. In this regard, a recent US study estimated lifetime risk of PAD by pooling 6 community-based US cohorts. According to that study, the lifetime risk of PAD was estimated to be ≈30% in Black men and women and ≈20% in White and Hispanic women and men. The study demonstrated that, for a given age, sex, and race/ethnicity, the lifetime risk estimate of PAD can vary by 3- to 5-fold depending on the status of the traditional risk factors for PAD such as smoking and diabetes.

DIAGNOSIS

Physiological testing

ABI, the ratio of ankle-to-brachial systolic blood pressure, is the first-line noninvasive diagnostic method for PAD, requiring standardized measurement methodology [6]. An ABI ≤0.90 is considered PAD. The diagnostic performance of ABI to detect PAD, with >50% stenosis based on imaging modalities as the gold standard, is reasonably good, with sensitivity and specificity, respectively, at 61 to 73% and 83 to 96%.

Several studies have shown that women tend to have lower ABIs than men, potentially because of shorter height [8, 9]. A population-based study specifically explored this issue and found that, after accounting for demographic and clinical factors (eg, age and height), healthy women had on average an ABI 0.017 lower than healthy men. Nonetheless, given the small difference from a clinical perspective for individual diagnosis, major clinical guidelines use the same ABI threshold of 0.90 in both sexes [1, 2, 20].

The ABI can be falsely high in the presence of stiffened ankle arteries related to medial artery calcification, a condition mostly observed in patients with diabetes or chronic kidney disease (CKD). In this scenario, it is recommended to measure the toe-brachial index (TBI), the ratio of the toe-to-brachial systolic blood pressure [1], because medial calcification rarely affects digital arteries (detailed techniques to measure TBI can be found elsewhere). In general, a TBI ≤0.70 is accepted as diagnostic for PAD [1, 2].

An ABI 0.90 to 1.0 is considered as borderline low ABI and cannot rule out PAD [8]. As detailed later in the statement, a body of evidence indicates that borderline low ABI is associated with increased risk of mortality and reduced physical function. In the case of borderline low ABI, particularly if symptoms suspect for exertional leg ischemia are present, the sensitivity to detect PAD can be improved by measuring ABI after a treadmill test (heel raise is an alternative method) [3, 4, 9, 20]. Although the criteria to evaluate postexercise ABI have not been standardized, postexercise ABI <0.90 or a drop of ABI >20% or ankle pressure drop >30 mm Hg are usually considered as diagnostic [10]. Postexercise ABI should be also considered in patients with potential intermittent claudication with normal ABI.

Another option to overcome ABI limitations is to study the ankle arteries' Doppler flow pattern and velocities. In my personal investigation of patients, the addition of tibial artery Doppler assessment identified 20% additional diseased legs missed by the ABI. Waveform analysis enables us to detect occlusive disease despite calcified arteries in patients with diabetes, and to identify those at high risk of cardiovascular disease (CVD) and limb events [3, 4].

Imaging

Noninvasive imaging for the assessment of anatomy and severity of arterial stenosis for patients with PAD has evolved over the past decade because of technical improvements [11]. These include the ability to image distal vessels with calcification, lower contrast dose, and higher spatial resolution. The selection of imaging modalities to diagnose PAD should depend on several factors, including the patients' symptoms (eg, claudication versus CLI), kidney function, and ABIs.

COMPUTED TOMOGRAPHIC ANGIOGRAPHY

Multidetector computed tomography scanners, including helical and multistation axial acquisitions, have now enabled the rapid scanning of the entire arterial system [12]. For evaluating the indication of revascularization in patients with PAD, both computed tomographic angiography (CTA) and magnetic resonance angiography (MRA) are accepted as appropriate imaging tests. The sensitivity and specificity of multidetector CTA compared with angiography is ≈90% for detecting PAD [13]. CTA uses iodinated contrast and ionizing radiation to visualize pathology from the aorta to the lower extremity. The scan times take a few seconds, but diagnosis can be difficult in small tibial vessels with calcification and multiple occlusions. The recent development of 256-row CTA has made detecting stenosis in the tibial location possible [13], except in patients with calcified disease. New imaging techniques are being developed, including computed tomography perfusion to allow visualization of hypoxic regions of the lower extremity [14, 15], which can also demonstrate the effect of interventional treatment [7, 26, 31].

MAGNETIC RESONANCE ANGIOGRAPHY

The sensitivity and specificity of MRA in detecting PAD with stenosis >50% is the same as CTA, 90 to 100% [35]. MRA has several advantages in diagnosing PAD over CTA. MRA requires no radiation, calcium does not interfere with the diagnosis, and it can be helpful in evaluating for bone marrow edema in patients who have ulcers with possible osteomyelitis. However, the procedure time is considerably longer. Also, there is a concern of gadolinium-induced nephrogenic systemic fibrosis in patients with decreased kidney function. Also, noncontrast MRA can be an option in some patients in capable facilities [16]. Another advantage of MRA is that it allows for hemodynamic measurements. Advanced techniques such as blood oxygenation level–dependent imaging and arterial spin labeling allow for assessing changes in perfusion to the calf muscle without gadolinium.

DUPLEX ULTRASOUND

This modality is safe to all patients but is operator dependent. The sensitivity and specificity depend on several factors, including the presence of calcium in the arterial wall, the location or depth of the vessel, and the presence of multiple occlusions at different locations [16, 17]. The femoral and popliteal arteries can usually be assessed well, whereas the iliac vessels and aorta can be challenging because of the presence of bowel gas and body habitus. This modality can also take some time to perform a complete examination [6]. Ultrasound is often used to assess the effectiveness and patency after endovascular and surgical treatment. New advances using contrast-mediated ultrasound are being developed to evaluate perfusion to the lower extremity [17].

Catheter-based angiography

Catheter-based angiography remains the gold standard for diagnosing PAD but is now limited to patients receiving endovascular revascularization [1]. New techniques are available that help to reduce the use of iodinated contrast, where CTA and MRA imaging can be fused to the angiogram, which has the potential to reduce the use of contrast and radiation [19]. Also, in some institutions, CO_2 angiography is used as a replacement or supplement (to reduce contrast) of conventional contrast-based angiography.

RISK FACTORS

Conventional risk factors

Evidence has supported traditional cardiovascular risk factors in PAD such as diabetes, smoking, dyslipidemia, and hypertension. A sedentary lifestyle also increases the risk in the development of PAD. The Edinburg study reported that the risk of PAD is inversely related to physical activity. Of these conventional risk factors, diabetes and smoking are particularly strongly related to the development of PAD.

Individuals with diabetes are at an increased risk of developing asymptomatic or symptomatic PAD, with an increase in claudication of 2- to 3-fold greater compared with individuals without diabetes. Diabetes worsens outcomes in patients with PAD, by mostly affecting infrapopliteal arteries, increasing risk of CLI, amputation, and mortality [27]. Accordingly, ≈70% of nontraumatic lower extremity amputations occur in patients with diabetes, disproportionally to its overall prevalence of 12% [28, 30].

From another perspective, PAD is an important contributor to diabetes-related foot ulcer, a devastating condition with a high mortality risk and high medical cost affecting ≈13% of patients with diabetes in the United States [25, 27]. Up to half of patients with diabetes-related foot ulcer have PAD [28, 29]. The presence of PAD significantly worsens the prognosis in patients with diabetes-related foot ulcer with decreased healing rates, recurrence of ulceration, major limb amputation, and long-term survival.

Like diabetes, cigarette smoking doubles the risk of PAD compared with nonsmoking. The risk increases cumulatively with the number of cigarettes smoked and the start age of tobacco use, with starting before 16 years of age having the greatest risk [32, 33]. Although smoking cessation decreases the risk of PAD, a recent community-based cohort study demonstrated that it takes up to 30 years for the risk for PAD of the individuals who stopped smoking to reach that of individuals who do not smoke, whereas the risk for coronary heart disease returns to the baseline within 20 years (Fig. 1).

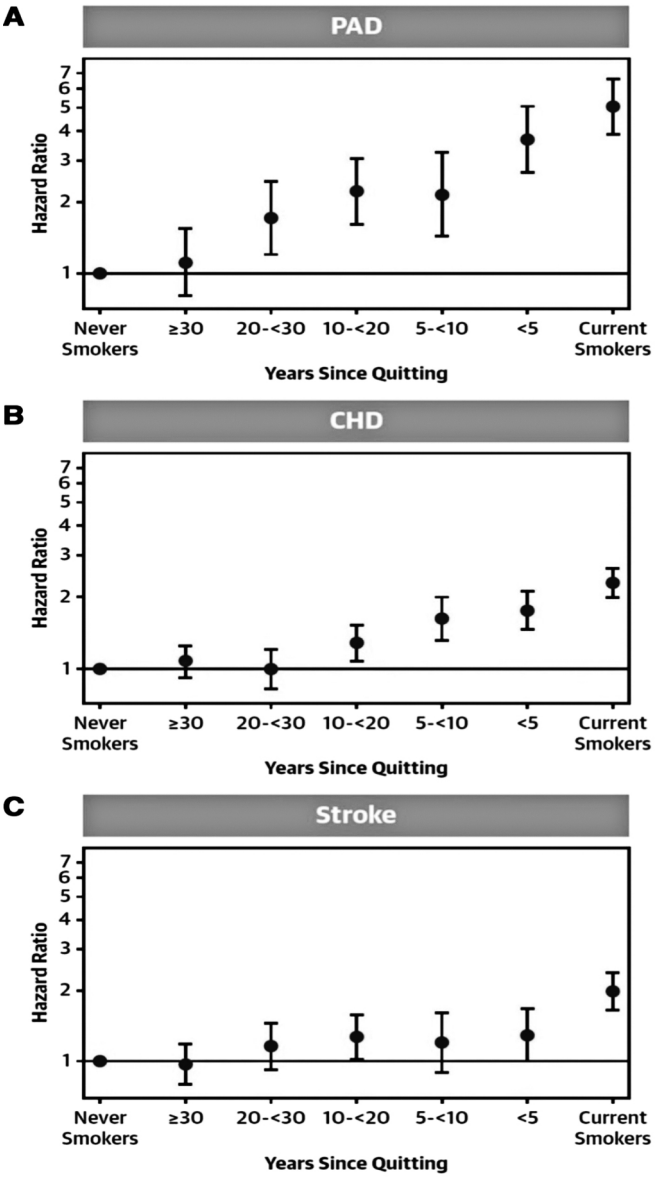

Fig. 1. Adjusted hazard ratio of 3 major atherosclerotic diseases according to time since quitting smoking: A — peripheral artery disease (PAD); B — coronary heart disease (CHD); C — stroke

Рис. 1. Скорректированный коэффициент опасности трех основных атеросклеротических заболеваний с момента прекращения курения: A — болезнь периферических артерий (PAD); B — ишемическая болезнь сердца (CHD); C — инсульт

Several studies have demonstrated total cholesterol and low levels of high-density lipoprotein cholesterol to be associated with PAD. In addition, apolipoprotein B and lipoprotein(a) levels have been shown as independent risk factors. A recent trial in patients with established CVD treated with hepatocyte-directed antisense oligonucleotide revealed a dose-dependent reduction of lipoprotein(a) [34], although the risk reduction of CVD including PAD is yet to be determined. A recent study has shown that triglyceride-rich lipoproteins may be especially important in the development of PAD. This observation has a clinical implication because icosapent ethyl, a triglyceride-lowering medication, has reduced major adverse cardiovascular events in REDUCE-IT (Reduction of Cardiovascular Events with Icosapent Ethyl-Intervention Trial) [37], although this trial has not reported results for PAD as an outcome.

Nonconventional risk factors

PAD develops as an inflammatory cascade within arterial walls leading to atherosclerosis. In the Edinburgh Artery Study, inflammatory markers such as CRP (C-reactive protein) and IL-6 (interleukin-6) were found to be elevated in patients with symptomatic PAD. Studies have found that elevated levels of these inflammatory markers are associated with the most severe form of PAD and at the highest risk for CVD events. Hemostatic factors such as fibrinogen have been associated as an independent risk factor [36] and a strong predictor for the development of PAD.

Some studies suggest HIV as a risk factor for PAD. A US study including veterans showed that individuals with a sustained CD4 cell count <200 cells/mm^3 had nearly 2-fold higher risk of PAD than individuals without HIV. There was no excess risk among individuals with a CD4 cell count ≥500 cells/mm^3.

There is an evidence demonstrating an association between metals and cardiovascular disease [38]. Despite mounting evidence, the relationship is underappreciated. For instance, lead exposure has been shown to contribute to 10 times the number of cardiovascular deaths originally estimated. The association of blood lead and PAD in National Health and Nutrition Examination Survey 1999 to 2000, revealed that blood lead levels were 14% higher in cases with PAD than without. The Strong Heart Study evaluated the association of urine cadmium concentrations with the incidence of PAD, showing a prospective association between PAD and urine cadmium, independent from smoking. Higher urine cadmium levels have been associated with an increase in PAD severity, with no PAD having the lowest urine cadmium concentration and CLI with the highest levels of urine cadmium [39].

Air pollution exposure is linked with CVD, including PAD [39]. A population-based study of 18 000 individuals, associated urban living with a 2- to 3-fold increased risk of PAD compared with individuals living in rural areas. Similarly, those living near major roadways demonstrated a decrease in ABI [39].

Depression has emerged as a risk factor for the incidence and progression of PAD. This may be attributable to medication noncompliance or a decrease in physical activity. The Heart and Soul study revealed a hazard ratio of 2.09 (95% CI, 1.09–4.00) of developing PAD in patients with depressive symptoms after adjustment for sex and age [39]. Individuals

with depression and PAD had worse functional outcomes, greater need for revascularization, and worse quality of life [40].

Microvascular abnormalities

PAD is usually recognized as a manifestation of macrovascular disease. However, several recent studies have indicated the potential involvement of microvascular disease in the progression of PAD. For example, an international consortium of individual-level data including 0.8 million adults has shown that albuminuria, a representative measure of microvascular disease, is more strongly associated with leg amputation than overall PAD (eg, adjusted hazard ratio ≈6 versus ≈3 in urinary albumin-to-creatinine ratio >300 versus <10 mg/g) [40, 42]. Moreover, a community-based cohort has demonstrated that the presence of any retinopathy (eg, hemorrhage or exudates) was more strongly associated with the incidence of CLI and PAD than that of coronary heart disease or stroke.

These observations have important diagnostic and therapeutic implications. For example, the ABI, which reflects stenosis in relatively large arteries, may not be helpful to classify the risk of CLI or leg amputation in some patients. A small case series has reported wide distribution of ABI (ranging from 0.7 to 1.1) in patients with diabetes and CLI. Of note, this study has demonstrated that all patients had TBI <0.7. Also, the current therapeutic options for patients with PAD (eg, statins and antiplatelets) are mainly based on evidence to prevent large artery disease or macrovascular disease (ie, coronary heart disease and stroke). Thus, future investigations on any therapeutic options targeting microvascular disease would be warranted.

COMPLICATIONS/COMORBIDITIES

Leg Symptoms, Physical Function, and Quality of Life

The magnitude and significance of functional impairment in PAD is underappreciated. Despite difficulty walking long distances, individuals with PAD frequently have atypical leg symptoms that can be mistaken for comorbidities such as hip or knee arthritis or spinal stenosis. Some clinicians may attribute difficulty walking to normal aging. Some people with PAD report no exertional leg symptoms (ie, are asymptomatic) either because they have restricted their physical activity or slowed their walking speed to avoid ischemic leg symptoms. Therefore, it is important for clinicians to suspect the possibility of PAD in people who report difficulty in walking because of discomfort, weakness, cramping, or other symptoms in the hips, lower extremities, or feet. This is particularly the case if the symptoms resolve with rest and do not begin with rest and if the patient is >55 years of age with cardiovascular risk factors or a history of other cardiovascular disease. Cilostazol is the sole medication that the AHA/ACC PAD guideline recommends for ameliorating leg symptoms and improving walking distance in patients with PAD [1].

The gradual but progressive nature of functional decline in PAD is also difficult for clinicians to detect without objective testing. Furthermore, patients with PAD who restrict their activity to avoid leg symptoms may not appreciate that their walking endurance has declined and may report stabilization of leg symptoms even as their 6-minute walk distance has declined [35, 41]. A 6-minute walk test can be used to measure objective change in walking ability. Greater declines in 6-minute walk distance over time are associated with adverse outcomes, including mortality and mobility loss.

Atherosclerotic obstructions in lower extremity arteries prevent delivery of oxygenated blood to lower extremity skeletal muscle during walking activity, and many people with PAD cannot walk >2 to 3 blocks without stopping to rest because of ischemic leg symptoms such as cramping, weakness, or pain. It is important for health care providers to acknowledge patterns of atypical symptoms in patients with PAD [18, 21]. For example, hip, buttock, and lower back pain that occur with walking and resolve with rest are common in people with PAD and are likely attributable to atherosclerotic disease in locations proximal to the femoral arteries.

Consistent with the phenomenon of walking-induced ischemia, people with PAD have lower physical activity levels, poorer walking endurance, slower walking velocity, and poorer balance than people without PAD. More severe PAD is associated with lower physical activity levels and greater functional impairment. In the Walking and Leg Circulation Study cohort of 460 participants with PAD and 240 without PAD, lower ABI was progressively associated with a higher odds ratio of stopping to rest during a 6-minute walk test (eg, 11.7 [95% CI, 4.9–27.7] in ABI <0.50 and 6.6 [95% CI, 3.1–14.1] in ABI 0.50 to <0.70 compared with participants with ABI 0.9–1.5).

People with asymptomatic PAD also have significantly poorer functional performance than those without PAD. In 2 large observational studies of older community-dwelling men and women, ≈65% of those with an ABI <0.90 consistent with PAD were asymptomatic (ie, reported no exertional leg symptoms). Yet these individuals with asymptomatic PAD still had significantly slower walking velocity, lower physical activity, and poorer walking endurance than people without PAD who also report no exertional leg symptoms. Of note, borderline low ABI 0.9 to 1.0 has also been independently associated with reduced physical function.

In addition to poorer performance on objective assessments of functional performance, people with PAD report poorer quality of life than those without PAD. In the ARIC Study with 5115 older adults, lower ABI was independently associated with lower quality of life. The association was more evident for physical domains than mental domains of quality of life. This pattern was consistently

observed in other studies. Nonetheless, in a study of 957 patients with PAD presenting to 16 specialty clinics in the United States, Netherlands, and Australia, 336 (35%) had significant mental health concerns consisting of depressive symptoms, anxiety, and stress.

Despite the significant functional impairment and impaired quality of life, people with PAD have traditionally been considered to have a benign natural history with regard to lower extremity outcomes [23]. This is because relatively few people with PAD will develop CLI or require amputation [31, 41]. The gradual decline in walking performance may be less perceptible to patients and to clinicians than acute events such as ALI, creating a false perception of a benign natural history of lower extremity PAD.

Leg outcomes (CLI/ALI, leg amputations)

Lower extremity major amputations (typically defined at the level of the ankle or above) and ALI are often considered major adverse limb events. Amputation is not simply a complication but an important treatment option to save lives and proximal limbs. The association of PAD with mortality and other cardiovascular outcomes like myocardial infarction and stroke has been extensively evaluated. However, few studies have quantified the association of PAD (versus no PAD) with severe leg outcomes, although several clinical studies are exploring those outcomes only among PAD patients [35]. There are no validated models to identify patients with PAD who are likely to develop CLI or need amputation. To the best of our knowledge, whether ABI is associated with future CLI or leg amputation in the general population has yet to be reported.

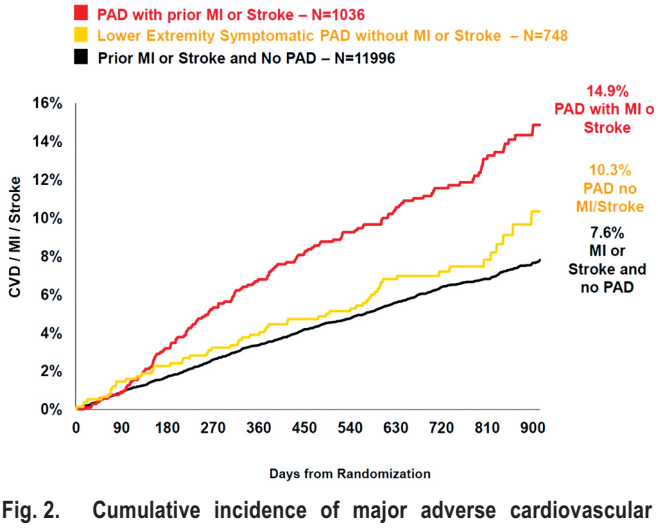

Fig. 2. Cumulative incidence of major adverse cardiovascular events in the placebo group according to CVD status at baseline. CVD indicates cardiovascular disease; MI — myocardial infarction; PAD — peripheral artery disease

Рис. 2. Кумулятивная частота основных неблагоприятных сердечно-сосудистых событий в группе плацебо в соответствии с состоянием CVD на базовом уровне. CVD указывает на сердечно-сосудистые заболевания; MI — инфаркт миокарда; PAD — заболевания периферических артерий

ALI is a vascular emergency requiring immediate treatment for limb salvage and has recently attracted attention as an important complication of PAD. ALI usually represents a rapid or sudden (eg, <2 weeks) decrease of leg perfusion causing pain, pulseless, pallor, sensory loss, or paralysis. However, to efficiently establish evidence on ALI, the field needs to develop a standardized definition of ALI [41].

Mortality and cardiovascular outcomes

The ABI Collaboration reported a robust association of a low (≤0.90) and high (>1.40) ABI with all-cause and cardiovascular mortality from a meta-analysis of 16 population-based cohort studies. In persons with an ABI between 0.81 and 0.90, total mortality was doubled and in those with an ABI ≤0.70 it was quadrupled. In this study, borderline low ABI also demonstrated significantly elevated mortality. Multiple studies in diverse populations have demonstrated that persons with PAD have higher risk of other CVDs such as coronary heart disease, stroke, and abdominal aortic aneurysm [1, 39]. Another study adds heart failure to these outcomes. The elevated CVD risk has been shown to be only partially attributable to shared CVD risk factors, such that at any given level of CVD risk factors, PAD is independently related to future CVD events and mortality. PAD has also been shown to be predictive of future CVD events even when adjusted for other markers of subclinical atherosclerosis [40].

PAD recently gained attention in the context of polyvascular disease. This refers to a subset of patients with atherosclerotic involvement of multiple vascular beds, including PAD. In several trials assessing new lipid-lowering or antithrombotic therapies in the field of cardiovascular prevention such as the FOURIER trial (Further Cardiovascular Outcomes Research With PCSK9 Inhibition in Patients With Elevated Risk) and the COMPASS trial (Cardiovascular Outcomes for People Using Anticoagulation Strategies), patients with polyvascular disease demonstrated higher risk than those without, which was translated into higher absolute risk reduction with these new treatments. For example, in the FOURIER trial, as anticipated, PAD plus myocardial infarction/stroke had the highest risk of major adverse cardiovascular events (CVD mortality, myocardial infarction, and stroke), with 2.5-year risk of 14.9% (Fig. 2). It is notable that PAD without myocardial infarction/stroke had a higher risk of major adverse cardiovascular events (10.3%) than myocardial infarction/stroke without PAD (7.6%).

CHALLENGES IN PAD MANAGEMENT

Underutilization of evidence-based preventive therapy

The most recent PAD guideline was developed in 2016 [1] and lists antiplatelet therapy, statins, antihypertensive

agents, glycemic control, and smoking cessation as the Class I (strong) and IIa (moderate) recommendations. Despite these evidence-based guideline recommendations, patients with PAD remain undertreated. In an analysis of persons with PAD (defined by ABI ≤0.9) from the National Health and Nutrition Examination Survey, the use of aspirin, statins, and renin-angiotensin system inhibitors was only 35.8, 30.5 and 24.9%, respectively. A more contemporary study of patients undergoing peripheral revascularization, a subgroup at heightened risk for cardiovascular and limb ischemic outcomes, reported use of aspirin, $P2Y_{12}$ inhibitor, and renin-angiotensin system inhibitors in 67.3, 57.7 and 47.6% of patients, respectively, at discharge. In the latter analysis, only 61.7% of patients were discharged on a statin. Provider efforts to help patients with smoking cessation were examined among 1272 patients with PAD cared for in vascular specialty clinics followed in the PORTRAIT Registry (Patient-Centered Outcomes Related to Treatment Practices in Peripheral Arterial Disease: Investigating Trajectories). In this study, 37.3% (n=474) were smoking actively at baseline. Of these, only 16% were referred to smoking cessation counseling, and 11% were prescribed pharmacological treatment. At 12 months, 72% of all individuals who smoked at baseline continued to smoke. The illustrated underutilization of preventive therapies may reflect the lack of clarity regarding prevention goals in PAD, because many trials have included PAD as a minority subgroup of broader atherosclerotic CVDs such as coronary heart disease and stroke. Nonetheless, these data clearly highlight the need for efforts to improve the use of evidence-based therapies in patients with PAD.

Underutilization of supervised exercise therapy

Supervised exercise is first-line therapy to improve walking impairment in people with PAD. Supervised treadmill exercise is the most thoroughly studied exercise therapy for people with PAD. More than 30 randomized clinical trials of supervised treadmill exercise in people with PAD involving >1400 participants have been completed. In 1 meta-analysis, mean improvement in treadmill walking distance was 180 meters and mean improvement in pain-free walking distance was 128 meters, compared with a nonexercise control group. Supervised exercise also significantly and meaningfully improves 6-minute walk distance and health-related quality of life in people with PAD. Several randomized trials have also demonstrated that arm and leg ergometry exercise, respectively, each significantly improve walking distance in people with PAD.

Structured home-based walking exercise interventions receive Class IIa recommendations in the AHA/ACC 2016 PAD guideline and have the potential to overcome some barriers of supervised exercise programs. However, home-based walking exercise interventions have had mixed benefits for improving walking ability in people with PAD [43, 44]. Three randomized trials of home-based walking exercise significantly improved walking ability, measured by 6-minute walk distance and treadmill walking performance, compared with a control group that did not exercise. These effective interventions have required periodic visits to the medical center for in-person coaching and feedback. A 6-month home-based exercise intervention that included weekly on-site visits to the medical center while helping patients with PAD adhere to walking exercise at home improved the 6-minute walk distance by 52 meters relative to a control group. In contrast, a 9-month randomized trial of home-based exercise that primarily relied on telephone calls, tapering to once per month did not show significant benefit compared with usual care. Although home-based exercise interventions can significantly and meaningfully improve 6-minute walk distance, it is important to keep in mind that the most effective interventions have incorporated regular visits to the medical center. A recent randomized clinical trial of home-based exercise in 305 participants with PAD demonstrated that exercise at an intensity that induced ischemic leg symptoms, but not exercise conducted at a comfortable pace without ischemic leg symptoms, significantly improved walking performance [44].

CHALLENGES IN REVASCULARIZATION

Revascularization for intermittent claudication

Guidelines from the AHA/ACC [1] and the Society for Vascular Surgery [22] recommend best medical treatment as the first-line treatment for claudication, with revascularization reserved for only refractory cases. These recommendations are based on data showing that there is a relatively low likelihood of limb loss associated with mild PAD and that long-term improvements in symptomatology may be limited. For example, recent data from the Invasive Revascularization or Not in Intermittent Claudication trial demonstrated that, after 5 years of follow-up, revascularization for claudication lost any early benefit and did not result in long-term health-related quality of life compared with best medical therapy. Despite guidelines recommending medical management as the first-line therapy for claudication, recent registry data from the Vascular Quality Initiative demonstrate that 27% of all open bypass procedures and even a higher percentage of endovascular interventions are performed for claudication. It is possible that many of the patients undergoing revascularization for claudication experienced severe claudication symptoms and that conservative management failed. For instance, in the CLEVER study (Claudication: Exercise Versus Endoluminal Revascularization) [45], the revascularization group and the supervised exercise therapy group had better 18-month outcomes than optimal medical care alone. Quality improvement initiatives aimed at reducing unnecessary procedures are emerging to address outlier behavior in the overuse of invasive interventions for

mild disease [47]. Higher-quality data about the benefits of revascularization for severe claudication symptoms are needed.

PERCUTANEOUS REVASCULARIZATION

The impact of percutaneous intervention in CLI is a subject of emergent research and the focus of active investigation. In a large observational study, percutaneous intervention compared with surgical therapy was associated with reduced in-hospital mortality (2.34% versus 2.73%, *P*<0.001), length of stay (8.7 days versus 10.7 days, *P*<0.001), and cost of hospitalization ($31 679 versus $32 485, *P*<0.001) despite similar rates of major amputation (6.5% versus 5.7%, *P*=0.75) [5]. Also, the increase in percutaneous leg revascularization has been related to a decline in leg amputation in the United States [5]. Although many observational studies have suggested the benefit of percutaneous intervention in decreased amputation rates and mortality, to date, only one trial has compared percutaneous intervention with medical or surgical therapy in patients with CLI.

Furthermore, most studies to date have failed to account for anatomic factors that may influence patient selection toward percutaneous versus surgical intervention. The Society for Vascular Surgery has developed 2 limb-staging classification schemes to allow for more objective comparison of revascularization outcomes. The Wound, Ischemia, and foot Infection (WIfI) stage [48] and the Global Anatomic Staging System (GLASS) are 2 classification systems intended to permit more meaningful analysis of outcomes for various forms of therapy in heterogeneous populations with CLI and should be reported whenever possible in major comparative studies moving forward.

With the increased use of percutaneous intervention in PAD, restenosis has been a continual obstacle. A growing proportion of patients are undergoing lower extremity bypass for a prior failed percutaneous intervention, and these secondary revascularization procedures have been associated with inferior 1-year outcomes. Although many devices lack comparative proof to support their use as a definite approach, multiple randomized studies of drug-eluting stent or drug-coated balloon show promising results for decreasing restenosis rates in the femoral-popliteal segment. Among the current therapeutic options, the paclitaxel-eluting or paclitaxel-coated devices consistently show a significantly higher primary patency rate, better target lesion revascularization rate, and cost effectiveness. Although a meta-analysis has reported an increase of mortality in patients receiving paclitaxel drug-coated balloon/drug-eluting stent DES compared with controls, there is some recent evidence against this finding. Nonetheless, the continued use of these devices should be individualized, carefully balancing the risks and benefits.

SURGICAL REVASCULARIZATION

The majority of open surgery for lower extremity revascularization is performed for CLI [18, 21, 26]. Although lower extremity revascularization for PAD is becoming increasingly common in the Russian Federation and all around the world, the rate of open surgery is stable or declining [31, 35, 41]. Approximately 40% of all lower extremity revascularization procedures performed in the Russian federation are open bypass surgery (versus 60% endovascular) because of the lower morbidity associated with endovascular procedures [46,48].

However, there is still substantial debate about the efficacy of open surgery versus endovascular interventions for the treatment of PAD. In the BASIL trial (Bypass versus Angioplasty in Severe Ischemia of the Leg), which is the only randomized controlled trial on the topic to date, a bypass-first strategy had overall outcomes similar to an angioplasty-first strategy [24]. However, there was a significant overall survival benefit and a trend toward a benefit for amputation-free survival associated with open surgery among patients who survived >2 years. Since that trial concluded >15 years ago, there have been major advances in endovascular technology that are associated with better long-term outcomes at higher costs. As a result, the efficacy of endovascular versus open surgery revascularization for PAD remains unknown. The BEST-CLI trial (Best Endovascular vs Best Surgical Therapy for Patients with Critical Limb Ischemia), which just completed enrollment, will hopefully clarify optimal therapies for CLI [31]. As noted earlier, the application of objective anatomic staging systems such as WIfI or GLASS are necessary to equalize clinical and anatomic factors in addition to baseline patient risk factors in clinical trials and observational studies moving forward.

Lower extremity PAD is a global public health issue that has been systematically understudied and underappreciated. This statement summarizes major gaps in research, clinical practice, and implementation related to PAD. Health care professionals, researchers, expert organizations, health care organizations, government agencies, industry, and the community should collaborate to increase the awareness and understanding of PAD and improve the quality of PAD diagnosis, management, prognosis and treatment.

ADDITIONAL INFORMATION

Author contribution. Thereby, all authors made a substantial contribution to the conception of the work, acquisition, analysis, interpretation of data for the work, drafting and revising the work, final approval of the version to be published and agree to be accountable for all aspects of the work.

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ДОПОЛНИТЕЛЬНАЯ ИНФОРМАЦИЯ

Вклад авторов. Все авторы внесли существенный вклад в разработку концепции, проведение исследования, прочли и одобрили финальную версию перед публикацией.

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией.

Источник финансирования. Авторы заявляют об отсутствии внешнего финансирования при проведении исследования.

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DEEP EUTECTIC SOLVENTS — A NEW METHOD FOR TRANSDERMAL DRUG DELIVERY

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Abstract. Transdermal drug delivery systems are more advantageous due to non-invasive treatment and pharmacokinetics, which makes / makes this method attractive in pediatric practice. Pharmacokinetic advantages consist of increasing bioavailability due to the absence of the effect of primary passage through the liver and uniform intake of the active substance into the systemic circulation, which is why the concentration curve of the medicinal substance is more uniform. Currently, plasters are used as transdermal delivery systems in the world, but the legal difficulties of commercialization of drugs for obstetric and pediatric audiences limit the possibilities of using transdermal therapeutic systems. In this review, transdermal drug delivery systems based on deep eutectic solvents are discussed. Due to unique properties such as ease of synthesis, low toxicity and cost, high stability and biocompatibility, deep eutectic solvents are attractive delivery systems for active pharmaceutical substances for use in pediatrics.

Key words: deep eutectic solvents; transdermal drug delivery; vaccine; active pharmaceutical substance.

ГЛУБОКИЕ ЭВТЕКТИЧЕСКИЕ РАСТВОРИТЕЛИ — НОВЫЙ СПОСОБ ТРАНСДЕРМАЛЬНОЙ ДОСТАВКИ ЛЕКАРСТВ

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Резюме. Системы трансдермальной доставки лекарственных средств имеют ряд преимуществ благодаря неинвазивному введению и особенностям фармакокинетики, что делает данный способ крайне привлекательным в педиатрической практике. Фармакокинетические преимущества заключаются в повышении биологической доступности из-за отсутствия эффекта первичного прохождения через печень, равномерного поступления действующего вещества в системный кровоток, благодаря чему кривая концентрации лекарственного вещества приобретает более равномерный характер. На данный момент в мире в качестве систем трансдермальной доставки используются пластыри, однако глобальные сложности правоприменительной практики вывода препаратов для акушерской и педиатрической аудитории на рынок лимитируют возможности создания транс-

дермальных терапевтических систем. В данном обзоре рассмотрены системы трансдермальной доставки лекарственных средств на основе глубоких эвтектических растворителей. Благодаря своим уникальным свойствам, таким как простота синтеза, низкая токсичность и стоимость, высокая стабильность и биосовместимость, глубокие эвтектические растворители являются привлекательными системами доставки активных фармацевтических субстанций для применения в педиатрии.

Ключевые слова: глубокие эвтектические растворители; трансдермальная доставка лекарств; вакцина; активная фармацевтическая субстанция.

For a few last decades transdermal drug delivery system (TDDS) has become the third common type of the drug delivery after oral drug introduction and injections. Popularity of transdermal drug delivery systems in the therapy is characterized by convenience of its application and pharmacokinetic peculiarities; taking into account that transdermal introduction is a preferable type of drug introduction for certain groups of patients. This cohort is represented by the patients with chronic pain syndrome when the uniform analgesic introduction maintains analgesic action; with the digestive system disorders, when the absorption of the drug can be disturbed in different parts of the gastro-intestinal tract and with those having difficulties in oral drug delivery, e.g. young children with maxillofacial, neck and mediastinum traumas and with dysphagia of different origins. It should also be noted that oral intake is limited by hydrolytic resistance issue caused by the active substance in acidic environment of the stomach and enzyme activity in the intestines; these substances can be delivered only parenterally.

TRANSDERMAL DRUG DELIVERY SYSEMS FOR ACTIVE PHARMACEUTICAL INGREDIENTS

Effective transdermal drug delivery systems development is relevant for the broad spectrum of active pharmaceutical ingredients. It is known that approximately 40% of available oral medications and 90% of new chemical compounds have low solubility and penetration via the skin which decreases their bioavailability when applied onto the skin [32, 51]. To solve the following issues different methods have been developed which were aimed at physical (dispersion), pharmaceutical and chemical modification of the active pharmaceutical ingredients. Increase of penetration due to chemical methods is achieved by interaction of such substances as water, hydrocarbon (alkanes and ethylenes), alcohol, acids, ether, alkyl esters of amino acids, amides, urea and its derivatives, amines and their bases, sulfoxides, terpens, steroids, dioxanes, pyrrolidone and imidazole derivatives, laurocapram (Azone) [41]. It results in: 1) changes in viscosity of the medication in the stratum corneum due to the lipid alkyl chain modifications; 2) increase of distribution coefficient of the ingredient on the skin; 3) drug reservoir formation on the outer layers of the skin associated with the formation of hydrophilic pores. However, relatively low number of the additional chemical substances (sulfoxide, terpenoid, glycoside, ethanol) are used in commercial drug production which increase transdermal penetration most commonly due to absence of toxicity and characteristic features of active substance interaction and also high cost of clinical trials. Thus, development and research of new biocompatible and biodegradable transdermal drug delivery systems for active medical ingredients in children is a relevant and modern task.

Present day methods of transdermal drug delivery system (TDDS) can be divided into two huge groups: transdermal delivery via active methods (the methods are associated with the drug delivery via ultrasound, current of the certain rate or laser) and transdermal delivery via passive methods (the methods are based on the application of the active pharmaceutical ingredient on different chemical substances or biological objects, which due to their specific functions can penetrate through stratum corneum: natural polymers, vesicles, nanoemulsions).

The active methods are sonophoresis, iontophoresis, electroporation, photomechanical waves, thermal ablation and microneedle drug delivery.

***Sonophoresis*.** A method of sonophoresis is based on low-frequency ultrasound which affects the outer layer of the skin cavitating the connective tissue and inhancing skin permeability. The medication is combined with gel or cream, which conduct ultrasound waves into the skin. Thus, the drug is delivered by the routes permitted by ultrasound with frequency ranging from 20 kHz to 16 MHz. The method allows to increase temperature on a localized part of the skin thus creating heat effect which contributes and enhances permeability of the medical substance [33;38].

The advantages of this method are:

1) Rapid penetration of the medical substance into the affected area and maximum concentration in it;
2) Prolonged effect of the medical substance which is stored in the tissues and released gradually afterwards;

3) Additional destruction of the clots.

The disadvantages may include:

1) More frequent medical procedures that are necessary when compared with the invasive methods;
2) Possible after-effects like tingling, irritation and burning sensations;
3) Inability to use the method when the stratum corneum is damaged.

Iontophoresis. This method is based on the use of the galvanic current of low-frequency, which can affect the outer and medial skin surfaces enhancing release and flow of the ionized active pharmaceutical substances which are characterized by low absorption / permeability. Efficiency of iontophoresis depends on polarity, valence and ability of the medical substance molecule to move (thermodynamic activity), nature of the applied electrical current and compositions of the agent which contains the medical substance [12, 13, 34, 38]. The advantages of the method are:

1) Delivery of polar molecules and high molecular weight compounds;
2) Targeted introduction of the medical substance into the affected area bypassing other organs which reduces allergic reactions and inflammations;
3) By hyperpolirising nerve endings it increases excitability threshold and enhances more analgesic effect.

The disadvantages may include:

1) Risk of burn if the electrodes are applied incorrectly;
2) Difficulty to control stability of the drug agent in the carrier;
3) Difficulty in drug release from the carrier;
4) It is contraindicated to the patients with cardiac pacemakers and metallic implants.

Electroporation. A method is based on application of electric impulses of high current ranging from 5 to 500 V. A short-term exposure of the skin (~ms) results in formation and opening of the minute pores in the stratum corneum. The medical substance is diffused through these pores. The method has confirmed its efficacy in drug delivery both with low-molecular weight such as doxorubicin, mannitol or calcein and high molecular weight anti-angiogenic peptides, oligonucleotides and negatively charged heparin anticoagulant [8, 49].

The advantages of the method are:

1) Muscle stimulating effect which improves tonus and blood circulation, it activates cell metabolism and increases skin rejuvenation;
2) High efficiency in targeted drug introduction.

The disadvantages are, that:

1) it can be used only in small areas;
2) there can be damage of the skin cells due to heating effect;
3) medical substance can be destroyed due to high voltage.

Photomechanical waves generated by the laser expose the skin and extend stratum corneum allowing the medical substances to pass through temporary channels. These waves produce limited ablation due to low radiation dose (nearly 5–7 J/cm^2), with the channel depth of 50–400 μm. For instance, dextran macromolecules with molecular weight of 40 kDa and latex particles with 20 ns can be delivered by photodynamic laser impulse 23 nm [27].

The advantages of the method are:

1) it transports the molecules of the medical substance through plasma membrane *in vitro*, thus preserving cell viability;
2) it does not affect the skin;
3) it is painless procedure.

The disadvantages are absence of clinical trials.

Microneedles. Micron sized needles penetrate the outer layer of the skin that results in drug diffusion through the epidermis or outer dermal layer. As the needles are short and fine the unpleasant pain sensations can be prevented, thus allowing the drug to be delivered directly to the blood capillaries. The microneedles can be: 1) microneedles that create a route allowing the drug to enter the body; 2) microneedles can be coated with the drug; 3) microneedles can be produced by the drugs which are absorbed in the body by “melting”; 4) different patches with microneedles [3, 16, 19, 23].

The advantages are:

1) painless introduction of the active pharmaceutical ingredient;
2) quick recovery of the injected area.

The disadvantages are:

1) only small doses of the substances can be used;
2) the secondary introduction decreases absorption in a certain topical area resulting from microclots and/or change in the blood flow at this site.

Thermal ablation. This method of the pointed distruction of the stratum corneum by local heat exposure delivers the drugs through the microchannels in the skin produced during the procedure. This method is based on exposure to high temperature more than 100 °C, resulting in heating and evaporating of keratonin. Thermal exposure lasts for microseconds and prevents epidermis damage. The defects resulting from the thermal ablation are small enough 50–100 mkm in diameter it allows to prevent painful sensations, bleeding, irritation and infection. The method allows delivering effectively small molecules and high molecular compounds. Thermal ablation can be performed by laser or high radiofrequency methods [3]. Laser thermoablation facilitates the drug delivery for more than 100 times and increases

delivery of both lipophilic and hydrophilic substances including peptides, proteins, vaccines and DNA. The radiofrequent thermal ablation method allows to release and deliver broad spectrum of drugs with hydrophilic origin, including macromolecules [25;37].

The advantages are:

1) the procedure is painless;
2) not expensive and disposable materials are used;
3) quick recovery.

The disadvantages are: the method is not recommended in hemostasis system disorders.

Passive methods are represented by (extracellular) vesicles, nanoparticles and nanoemulsions.

Vesicles are lipid bubbles which are secreted mostly by all the types of the cells. Being the carriers of RNA, membranous and cytoplasmatic proteins, lipids and carbohydrates they perform different functions in the human body, for instance, they take part in intracellular communication. According to their origin the vesicles are divided into ectosomes (originating from the neutrophils/monocytes), vecosomes (associated with the vector of adenovirus) etc. According to the biogenesis mechanism they are divided into exosomes, microvesicles and apoptotic bodies [14]. The size of the vesicles varies, for example the size of the exosomes is between 40–120 nm, microvesicles vary 50–1000 nm [4]. Due to such properties as biocompatibility, low immunogenicity (when obtained from the correct type of the cell) and ability to penetrate the blood-brain barrier (BBB) the vesicles appear to become a prospective mean to deliver different molecules.

The advantages are:

1) Controlled release of the medical substance;
2) Control of the drug absorption due to multileveled structure.

The disadvantages are:

1) Chemical instability;
2) High cost;
3) Restrictions associated with the volume of the drug upload.

Nanoparticles are represented by the nanocarriers with the size of 1–1000 nm. Introduction of the medical substance by means of nanoparticles leads to targeted and controlled release, changes in substance dynamics *in vivo* and increase of duration of the uploaded substance in the body which results in better bioavailability, decrease of toxicity and side effects. Nanoparticles can be polymerized or bound; most commonly biodegradable polymer materials like gelatin and polylactic acid are used [13, 18].

The advantages may be:

1) Targeted drug delivery;
2) Mechanical resistance of the carrier;
3) Different biodegradable materials can be used;
4) Both hydrophilic and hydrophobic substances can be delivered;
5) No immune reaction to the carrier.

The disadvantages are:

1) Difficulty for substance release;
2) Incomplete assessment of toxicity.

Nanoemulsions are represented by mixture with low viscosity, isothropic, thermodynamic and dynamic stability. Mixture is composed of transparent or semitransparent oil globules dispersed in water phase which is stabilized by interphase membrane which is formed due to molecules of the outer active substance. The size of the particles in nanoemulsions varies from 100 to 1000 nm. Due to their small size, significant specific surface area and low surface tension of nanoemulsions determine excellent wettability which allows close contact with the skin. Nanoemulsions demonstrate better properties of transdermal absorption than any of the commonly used local appliances [21, 36].

The advantages are:

1) Thermodynamic stability;
2) High solubility and physical stability.

The disadvantages are: variable kinetics of the processes of distribution and clearance of drug delivery (mechanisms).

DEEP EUTECTIC SOLVENTS

Deep eutectic solvents open promising perspectives of the controlled transdermal drug delivery. It is known that such solvents can penetrate the barrier of the stratum corneum and increase transdermal, intercellular and paracellular transport due to the cell integrity destroy, creation of the diffusion routes and solution of the lipid components of the stratum corneum [44, 47].

Deep eutectic solvents (DESs) were first described by Abbott et al [1]. If DES is composed of components of natural origin it is further described as natural deep eutectic solvent (NADES) [45]. DES/NADES are mixtures of two and more components, namely hydrogen bond acceptors (HBD) and hydrogen bond donors (HBD) which may compose eutectic mixtures characterized by very low melting temperature than in their components.

DESs have several advantages like thermal and chemical stability, fast solubility of the chosen substances, inflammability and low melting temperature. Moreover, DES can be obtained simply and cheaply by combination and heating natural and/or widely used substances. As a result these solutions are cheap, biodegradable with very low or lack of toxicity [52].

DES can be classified into several classes according to the nature of HBA and HBD which was used in their obtaining; thus, the salts of quaternary ammonium and anhydrous metal halides (type 1), the salts of quaternary ammonium and hydrated metal halides (type 2), the salts of quaternary ammonium and neutral organic compounds (type 3), salts of metal chlorides and neutral organic compounds (type 4) and mixtures of nonionic compounds (type 5) [2, 48]. Within these five types of DES the separate components can form binary or ternary eutectic mixtures.

NADES is a DES subgroup, which is composed of natural components like sugar, organic acids, (poly)alcohol, aminoacids, choline chloride and water. NADESs in terms of biological systems may serve as solvents which can be alternative to water and lipids, participate in biosynthesis, storing, transporting of poorly soluble in water biomolecules and conserving organisms at very low temperatures [10]. NADES turn to be highly attractive due to their low volatility; they are biodegradable, stable to the solved substances, resistant to air and simple in synthesis. One of the main advantages of NADES is that their properties can be modeled by changing their components, dissolving with water or synthesizing the targeted mixtures for certain purposes [20].

ChCl (Ch, 2-hydroxyethyl-trimethylammonium, vitamin B_4) is the most investigated and well-known component of DES which is quarternary ammonium salt and alcohol. In eutectic solvent mixtures ChCl acts as acceptor of hydrogen bond with different donors like urea, alcohol, sugar, hydroxyl acids and amino acids [45]. ChCl based DESs are very simple, their synthesis allows to control the characteristic features decreasing or increasing viscosity, pH and polarity; these properties make them attractable for pharmaceutical use, food production and cosmetology [11].

ChCl based DES can be grouped according to hydrogen donor relations: alcohol- and sugar containing, acidic, amidic, aqueous and triple mixtures. Most of such mixtures are liquid at a room temperature and can be used as solvents in various fields.

In alcohol- and sugar containing DES glycol, glycerin and different sugars are commonly used as HBD. These solvents have neutral pH in mixtures; it can be applied in various fields [31].

Acid based DESs are composed of natural carbon acids (lactic, lemon, wine etc.) and amino acids.

The mixture of ChCl and urea is one of the most well-known and investigated among amide DES with a ratio of 1:2 [1]. The synthesis of the triple mixtures by introducing of the third donor of hydrogen relation to DES is also possible. Most commonly water is the third donor. Besides water glycerin, methanol, ethanol, 2-propanal etc. can also be used [42].

DEEP EUTECTIC SOLVENTS AS SYSTEMS OF INSULIN DELIVERY

Insulin is a most common medication in the treatment of diabetes and diabetic complications. Nowadays insulin is introduced subcutaneously, such invasive method is rather painful for the patients; the clinicians and scientists look for non-invasive methods of diabetes therapy. A. Vaidya, S. Mitragorty (2020) used choline and geranic acid based solvents as transdermal system of insulin delivery and controlled release [50]. The medication had a form of viscous gel, which could be introduced perorally, the pharmacological effect of insulin was preserved. It was proved that the local introduction of insulin containing DES (insulin dose 25UN/kg) considerably reduced glucose in blood within 4 hours [24].

The method of nasal introduction of insulin based on DES (deep eutectic solvent was based on choline chloride mixture: malic acid) was studied, the method showed hypoglycemic effect [24]. The trial compared to systems of insulin delivery: on the base of hydrogel and on the base of deep eutectic solvents, it was proved that the system of DES insulin delivery was better than the system of hydrogen based insulin delivery and other traditional insulin solvents. The results show the possibility of deep eutectic solvents application as systems for insulin delivery in diabetes therapy.

Thus, it can be concluded that DES applications to be prospective carriers of insulin in the treatment of endocrine diabetes by means of introducing the mixtures transdermally, through mucous membranes of nasal and oral cavities.

DEEP EUTECTIC SOLVENTS AS SYSTEMS OF NON-STEROIDAL ANTI-INFLAMMATORY DRUG DELIVERY

Non-steroidal anti-inflammatory drugs (NSAIDs) are commonly prescribed medications to relieve pain syndrome and inflammation. The main effect of these medications is achieved by blocking specific prostaglandin synthesis through inhibition of cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) enzymes. The inhibition of COX-2 plays an important role in the anti-inflammatory mechanism and analgesic effect of the medications, besides these effects it can result in disorders of the cardio-vascular system. The disadvantages of the inhibition of COX-1 are in severe gastrointestinal ulceration and nephrotoxicity. If the side-effect on the gastrointestinal tract can be inhibited by phospholipids or simultaneous intake of stomach protecting medications (like proton pump inhibitors), there is still nothing to prevent heart and kidney side effects [17]. Taking into account that NSAIDs are commonly prescribed medications

the transdermal way of introduction seems to be alternative, effective, safe and psychologically comfortable during the course of treatment.

The trials showed that the drugs traditionally introduced intravenously can penetrate transdermally, too [15]. Thus, many kinds of NSAIDs have become available in various forms like creams, gels, patches and solutions (lotions), which are mainly prescribed to patients with muscular-skeletal pains. However, the production of such NSAIDs systems is complicated due to low water-solubility of the active ingredient, which requires highly concentrated organic solvents, e.g., ethanol.

In order to avoid organic solvents and improve delivery of poor water-soluble molecules deep eutectic solvents were investigated as alternative to pharmaceutical solvents and inhancers of transdermal penetration [6, 39]. It was proved that DES can improve solubility of anti-inflammatory drugs (e.g., ibuprofen, naproxen, ketoprofen [26] and paracetomol [29]), and also increase transdermal penetration of the drugs.

DEEP EUTECTIC SOLVENTS AS SYSTEMS OF ANTINEOPLASTIC (ANTITUMOR) DRUGS

In a case of malignant tumor, driver and "passenger" mutations result in significant alteration of signaling pathways which considerably change metabolism of the tumour cells. The area of tumor formation and character of bloodstream in tumor altered tissue form nucleus, lacking oxygen supply, and conditionally considered as a geometrical center of the neoplasm and/or metastases, where more resistant to nutrient and oxygen deficiency cell clones are selected, thus resulting in local evolution of signaling pathways and tumor cell metabolic reprogramming [30]. Thus metabolic pathways turn to appear most preferable targets in therapy of oncological diseases. For example, it was revealed that limonene induces apoptosis through mitochondrial pathway and affects survival potential / apoptosis of the cells passing through PI3K/Akt signaling pathway in a case of cholorectal cancer [5, 7].

Nowadays a significant number of antitumor drugs are synthesized, they affect different metabolic routes; nonetheless, the issues of selective drug delivery are at the cutting edge of science. There are scientific investigations when deep eutectic solvents were used to treat cancer due to their own antitumor activity or their ability to solve active pharmaceutical substances. It revealed that limonene-based DES (ibuprofen: limonene with a molar ratio of 1:4) can effectively inhibit HT-29 colonic cancer cell proliferation without affecting healthy cell viability [40]. The system could preserve not only the therapeutic effect of limonene and ibuprofen but also increased solubility of the both components reducing limonene side effect concerning healthy cell lines.

Betaine and mandelic acid-based DES was synthesized to deliver antineoplastic drugs perorally (cyclopeptide RA-XII). Solubility and bioavailability of RA-XII by peroral introduction increased 17.5 and 11.6 times respectively [28]. It should be underlined that choline and its metabolite betaine-based DES is of great interest, as these compounds participate in basic physiological processes: sustaining structural stability and membrane elasticity due to phosphatidylcholine formation during the metabolic process, acetylcholine synthesis and participation in homocysteine metabolism [28].

Cytotoxicity of N, N-diethylammonium chloride — based DES and choline chloride-based DES by interaction of these solvents and (HelaS3, AGS, MCF-7 и WRL-68) tumor cell lines were investigated with the help of the methods of molecular dynamics [35, 46]. As a result N,N-diethylammonium chloride-based DES showed higher cytotoxicity than choline chloride-based DES, it indicates that N,N-diethylammonium chloride-based DES is potent as independent anticancer drug.

Interesting results are represented by the research of P. Pradeepkumar et al (2021) [43]. The reserarchers developed serine and lactic acid-based DES, the obtained solvent and biotine were introduced to chitosan a polymer carrier. Then doxorubicin for controlled release was applied. HeLa cell line was the model of anticancer activity and *in vitro* apoptosis investigation.

DEEP EUTECTIC SOLVENTS AS SYSTEMS OF STORING AND VACCINE DELIVERING

The vaccines are usually stored at low temperature (2–8 °C) for stability and safety of vaccine efficiency. The vaccines require certain low temperature and they are at risk of unexpected sudden changes of storage; these factors motivate the researchers to find new systems to sustain increased stability that can prolong vaccine storage, facilitate its storage condition avoiding cooling. Moreover, the vaccination is invasive procedure though nasal introduction has become more popular recently. Both methods have great psychological load to small children. That is why new systems of storing at milder conditions as well as non-invasive drug introduction are urgent and relevant tasks.

The scientific investigation [22] performed DES to store human interferon-α_2 at room temperature, and to stabilize and carry the live attenuated vaccines [53]. The other investigation [9] demonstrated natural deep eutectic system application consisting of trehalose and glycerin to store and deliver the vaccine based on virus-like particles (VLP) and influenza hemagglutinin (HA). DES supported stability and

activity of HA-VLP from 4 to 50 °C (increased stability investigation). Moreover, HA-VLP were stable in the solvent for more than a month in an area with standard room temperature (short-term stability investigation).

These investigations show the prospectives in the field of temperature regimen changing in large protein molecules, vaccines and sera, that will allow cost and storage decrease in the near future.

CONCLUSION

The field of deep eutectic solvents application including pharmaceutical industry has considerably increased for the last decade; it is due to their unique characteristics as low toxicity, thermal and chemical stability, biodegradability and high bioavailability. These solvents can be used for solubility and stability of the systems of transdermal drug delivery and transdermal drug delivery system on their base it seems to be highly important for pediatrics. It should be noted that DES themselves have antibacterial, antifungul and anticancer properties. Due to specific properties of DES "smart" nanovaccines development seems to be attractive. The solvents may include modified nanoparticles to achieve controlled and prolonged transdermal immunization. Unlike the traditional method of invasive drug delivery DES allows to arrange a reservoir of medication in the skin thus prolonging duration of drug activity and showing low toxicity in transdermal immunization, absence of pain syndrome and psychological comfort in pediatric therapy.

Nonetheless, despite all advantages it is difficult to achieve stable and long lasting effect of medication release in most cases of drugs based on deep eutectic solvents; these combinations turn to be the variant for single noninvasive drug delivery. Various investigations to study kinetics of the solved drug release should be carried out to develop transdermal therapeutic systems. Moreover, law enforcement practice of clinical trials with drugs introduced transdermally in the form of gels and based on deep eutectic solvents should also be clarified.

ADDITIONAL INFORMATION

Author contribution. Thereby, all authors made a substantial contribution to the conception of the study, acquisition, analysis, interpretation of data for the work, drafting and revising the article, final approval of the version to be published and agree to be accountable for all aspects of the study.

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POST-TRAUMATIC STRESS DISORDER ASSOCIATED WITH PREGNANCY AND CHILDBIRTH: DEFINITIONS, MODERN CONCEPTS, PATHOPHYSIOLOGICAL MECHANISMS, RISK FACTORS, DIAGNOSIS

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Abstract. The review focuses on post-traumatic stress disorder associated with pregnancy and childbirth. In the literature, there is the concept of post-traumatic stress disorder associated with pregnancy and childbirth, which describes mental disorders in parents during the period from the beginning of pregnancy to 12 months after childbirth. In contrast to the classical concept of post-traumatic stress disorder, this term is used in most cases in relation to the mother, less often in relation to the father of the child, in the presence of traumatic events associated with pregnancy and childbirth, starting from the beginning of pregnancy and up to 1 year after childbirth. The uniqueness of this term is emphasized from the perspective of mental disorders in parents associated with traumatic events during pregnancy, childbirth and the subsequent fate and prognosis for a premature child and/or a child with pathology. Data is provided on the complexities of terminology used to describe traumatic events from the beginning of pregnancy to 12 months after childbirth. Information is presented on the prevalence of stressful conditions during the period associated with pregnancy and childbirth in parents, risk factors are described in detail, including obstetric, socio-economic, pathophysiological, and psychiatric. The stressful conditions of parents whose children are in the intensive care unit are described separately, with a description of the parents' reactions to different outcomes of the child's hospitalization. Options for the prevention of stressful conditions in parents are highlighted, and recommendations are provided for identifying patients suffering from post-traumatic stress disorder associated with pregnancy and childbirth, and for the interaction of maternity workers with this group of patients.

Key words: post-traumatic stress disorder; postpartum post-traumatic stress disorder; perinatal loss; postpartum period; traumatic birth.

ПОСТТРАВМАТИЧЕСКОЕ СТРЕССОВОЕ РАССТРОЙСТВО, СВЯЗАННОЕ С БЕРЕМЕННОСТЬЮ И РОДАМИ: ДЕФИНИЦИИ, СОВРЕМЕННЫЕ ПРЕДСТАВЛЕНИЯ, ПАТОФИЗИОЛОГИЧЕСКИЕ МЕХАНИЗМЫ, ФАКТОРЫ РИСКА, ДИАГНОСТИКА

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Резюме. Обзор посвящен посттравматическому стрессовому расстройству, связанному с беременностью и родами. В литературе существует понятие посттравматического стрессового расстройства, связанного с беременностью и родами, которое описывает психические нарушения у родителей в период от начала беременности до 12 месяцев после родов. В отличие от классического понятия посттравматического стрессового расстройства данный термин употребляется в большинстве случаев по отношению к матери, реже по отношению к отцу ребенка, при наличии травмирующих событий, связанных с беременностью и родами, начиная от начала беременности и включительно до 1 года после родов. Подчеркивается уникальность данного термина с позиции психических нарушений у родителей, что связано с травмирующими событиями на протяжении беременности, родов и дальнейшей судьбой и прогнозом для недоношенного ребенка и/или ребенка с патологией. Приводятся данные о сложностях терминологии, используемой для описания травмирующих событий от начала беременности до 12 месяцев после родов. Изложена информация о распространенности стрессовых состояний в период, связанный с беременностью и родами, у родителей, подробно описываются факторы риска, в том числе акушерские, социально-экономические, патофизиологические, психиатрические. Отдельно описываются стрессовые состояния родителей, чьи дети находятся в отделении реанимации и интенсивной терапии, с описанием реакции родителей при различном исходе госпитализации ребенка. Освещаются варианты профилактики стрессовых состояний у родителей, а также приводятся рекомендации по выявлению пациентов, страдающих посттравматическим стрессовым расстройством, связанным с беременностью и родами, взаимодействию с данной группой пациентов работников родовспомогательных учреждений.

Ключевые слова: посттравматическое стрессовое расстройство; послеродовое посттравматическое стрессовое расстройство; перинатальная утрата; послеродовый период; травматичные роды.

Posttraumatic stress disorder (PTSD) is defined as “a complex of somatic, cognitive, affective and behavioral consequences of a psychological trauma” [19, 20]. PTSD associated with pregnancy and childbirth (P-PTSD) is a type of PTSD, but it is a unique variety of the latter and it has common features with the classical PTSD, with which an average of 4 to 8% of the general population are affected at any given time. Classical PTSD is more common in women [6, 13, 17, 38].

PTSD associated with pregnancy and childbirth occurs after experiencing a traumatic event in women at any time after conception and up to 6–12 months after childbirth, it lasts longer than 1 month and has an extremely negative impact on the health of the mother and child [8]. From 3 to 15% of women experience PTSD during pregnancy and childbirth [8]. Approximately 3.3% of pregnant women suffer from PTSD and 4% of women suffer from postpartum PTSD [3]. According to a number of data, the incidence of pregnancy-related PTSD in women ranges from 2.3 to 24% [13]. Thus, the data on the prevalence of PTSD during pregnancy and childbirth vary.

Currently, the number of studies on PTSD during pregnancy and childbirth is limited. In the 1990s, the first studies aimed at postpartum PTSD began to appear, but none of the mothers met the criteria of classical PTSD [3, 6].

PTSD during pregnancy and after childbirth can be a continuation of pre-existing post-traumatic stress or reactivation of post-traumatic stress in remission [17].

Post-traumatic stress disorder associated with pregnancy and childbirth, at times with pronounced clinical manifestations, can also be observed in health professionals of obstetric institutions when they provide medical care in difficult situations involving a risk for a woman and her child, as well as if these factors are combined with personal psychological trauma [32].

PATHOPHYSIOLOGY OF POST-TRAUMATIC STRESS DISORDER ASSOCIATED WITH PREGNANCY AND CHILDBIRTH AND STRESS RESPONSE

In a perinatal loss, one of the factors in the development of post-traumatic stress disorder is the interruption of the chemically mediated connection between mother and child by reproductive hormones [8].

Post-traumatic stress disorder during pregnancy is associated with impaired regulation of cortisol, vasopressin and oxytocin. Disruption of the regulation of the latter can cause complications in childbirth [9, 16, 26].

Studies of post-traumatic stress have shown that memories of a traumatic event activate the amygdala and stimulate the emergence of a fear reaction in a person as the „fight-or-flight“ response, increasing the breathing and heart rates, increasing blood pressure and contributing to the shift of blood flow from visceral muscles to skeletal ones [14]. When adapting to dangerous situations, the „fight-or-flight“ response may become predominant by default with the activation of the hypothalamic-pituitary-adrenal axis, remaining in non-dangerous situations when exposed to the trigger [14].

There is a polyvagal theory of stress, also known as Stephen Porges' vagus nerve theory, according to which, in the case of stressful events, the sympathetic nervous system is involved through the hypothalamic-pituitary-adrenal axis by releasing catecholamines and the parasympathetic system — by releasing oxytocin, as a result of which a person tries to reduce the level of stress looking for a partnership with another person who does not experience a „fight-or-flight" response [14].

Upcoming childbirth can contribute to the activation of the „fight-or-flight" response, alter the functioning of the hypothalamic-hypophyseal-adrenal axis and the level of catecholamines [14]. The search for support among the environment and staff, mutual regulation of the sympathetic and parasympathetic systems can be a therapeutic response to stressful events through the anti-stress properties of oxytocin [14].

One of the theories of post-traumatic stress disorder is the failure of the fear reaction to subside [14]. Under the action of the trigger that causes the fear reaction, the activation of the hypothalamic-pituitary-adrenal axis continues, since the hippocampus and prefrontal cortex (which usually reduce the fear reaction in the absence of real danger) do not react in the usual way. The trigger-specific reaction in post-traumatic stress disorder is not sufficiently modulated [14]. A hypermodulated reaction similar to freezing or fainting is possible. To interrupt the fear reaction, it is necessary to use the cognitive processes that control the trigger and transform the provoking automatic repeated experiences and hypo- and hyperexcitation reactions [14, 17, 25, 36]. Current research shows that the use of cognitive behavioral therapy works by inhibitory learning when a person realizes that s/he can tolerate triggers, thereby weakening the habitual fear and avoidance reactions to reminders of the traumatic event [14, 25, 27, 36].

In addition, stress during pregnancy leads to the suppression of the placental enzyme 11-β-hydroxysteroid dehydrogenase type 2, which performs a protective function by blocking excess cortisol, which contributes to survival in difficult conditions, but has a negative impact on the further development of the child [14].

Post-traumatic dysregulation of oxytocin may result in pain syndrome, which is secondary to the dysregulation of smooth muscle peristalsis in patients with pelvic pain, irritable bowel syndrome, bladder pain. There is a cascade theory, according to which traumatic experiences in childhood, such as abuse, lead to a cascade of adaptive functions of oxytocin, catecholamines and to emergence of the hypothalamo-pituitary-adrenal axis, that may persist into adulthood [14].

Thus, there are a few distinct theories of postpartum affective disorders, such as postpartum depression and postpartum psychosis. However, as to post-traumatic stress disorder and stress reactions associated with pregnancy and childbirth, then changes in the level of hormones, genetic mechanisms, changes in the neuroimmune system cannot fully explain the stress reactions during pregnancy and after childbirth. This undoubtedly requires additional study of the pathophysiological mechanisms of stress during these periods. So the possible difficulties with working out clinical criteria and treatment of post-traumatic stress disorder associated with pregnancy and childbirth may result [26, 27].

CLINICAL PRESENTATION OF POST-TRAUMATIC STRESS DISORDER ASSOCIATED WITH PREGNANCY AND CHILDBIRTH. DIAGNOSTIC CRITERIA

1–2 months after childbirth, 33% of women experienced the so-called intrusion symptoms (obsessive memories, nightmares, somatic manifestations) associated with the stress during pregnancy and childbirth, 33% of women reported constant nervous tension, agitation [6]. Post-traumatic stress disorder associated with pregnancy and childbirth is characterized by depressive symptoms, suicidal thoughts, feelings of guilt, anger, immediate stress reactions, feeling of grief, obsessive thoughts and memories (often memories are vivid), a feeling of being retarded, a feeling of emotional numbness, avoidance of the child delivery reminders or other events related to the child, increased irritability, a feeling of losing control over one's life, a feeling of being trapped [4, 17, 36]. Concentration of attention is significantly reduced, the normal daily functioning of patients is disturbed, intra-family relationships and relationships with others can noticeably deteriorate [4, 12].

At present various researchers are trying to identify and summarize the criteria for postpartum post-traumatic stress disorder associated with pregnancy and childbirth, but doctors continue to be guided by the main criteria for post-traumatic stress disorder.

In the current international classification of diseases, post-traumatic stress disorder is classified under the heading F43 „Reaction to severe stress and adaptation disorders". Directly post-traumatic stress disorder is encoded with the code F43.1. Diagnostic criteria, according to ICD-10, are: an exceptionally strong, but short-lived (within hours, days) traumatic event that threatens the mental or physical integrity of the individual, an abrupt change in social status or environment [1].

DSM 5 (Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition) also describes the main symptoms of post-traumatic stress disorder: repetition of obsessive thoughts and dreams associated with a traumatic event, constant avoidance of any reminders of trauma, sleep disorders, irritability, outbursts of anger and aggression, negative changes in mood and thoughts [2, 5, 18].

It is worth noting that criteria for post-traumatic stress disorder such as a real threat to life, trauma, violence cannot be applied to post-traumatic stress disorder associated with pregnancy and childbirth, since most women have had no real threat to their, neither to the life or their newborn [5].

Thus, the main distinctive features of pregnancy and childbirth related PTSD are associated with such events as traumatic child delivery, death of the child or his or her stay in the resuscitation or intensive care unit.

In the clinical presentation of post-traumatic stress disorder associated with pregnancy and childbirth, typical manifestations are: repeated experiences of psychotrauma in the form of obsessive memories (reminiscences), such as intrusive memories of emergency caesarean section or hemorrhage, nightmare dreams, fantasies and visions; making up a background to repeated experiences of psychotrauma, there is a feeling of „numbness" and flattening of affect, social alienation, reduced reaction to surrounding people and events, anhedonia, avoidance of situations reminiscent of psychotrauma, avoiding the place where psychotraumatic events occurred; in rare cases, a woman can avoid her child, trying to spend less time with him/her, at times there may be acute episodes of fear, panic, aggression caused by unexpected memories of psychotrauma or reaction to it, a state of increased nervous tension, which, for example, is manifested by flinching at the crying of the child, insomnia. There is also increased autonomic excitability, increased wakefulness with insomnia, pronounced fright reactions. The onset of the disorder after a latent period is from a few weeks to six months [1, 2, 6, 8, 36].

In the clinical presentation of post-traumatic stress disorder associated with pregnancy and childbirth, there is a concept of internal and external distress. Externalization of distress consists in negative reactions aimed at other people. Such reactions seem to be caused by a feeling of despair or revenge. Internal distress reactions seem to occur in a person previously traumatized in his childhood. However, these manifestations do not occur in all people experiencing stress during pregnancy and after childbirth. Patients with behavioral disorders who exhibit aggression in obstetric institutions should be under constant supervision of medical staff [6].

In case of internalization of distress during pregnancy and after childbirth, patients tend to blame themselves for all events that have occurred, one can see psychosomatic manifestations, deterioration of general well-being, exacerbation of chronic diseases, possible short-term manifestation of these phenomena or long-term ones, up to several months or years. There are manifestations of anxiety and depression, the possible development of eating disorders such as anorexia nervosa, overeating, etc. [6, 22]. The prolonged course of maternal depression affects the mother–child dyad, affection that gets disturbed (insecure attachment), which also affects subsequent generations [3].

Thus, post-traumatic stress disorder associated with pregnancy and childbirth, although it is similar to the classic post-traumatic stress disorder, it has its own unique criteria related to pregnancy and childbirth, which should be taken into account when diagnosing, treating and working with this group of patients.

RISK FACTORS

Identification of risk factors makes it possible to prevent or reduce clinical manifestations of post-traumatic stress disorder associated with pregnancy and childbirth [8, 17].

CHARACTERISTICS OF THE CHILDBIRTH PROCESS

Risk factors include traumatic child delivery. Women, referring to the process of traumatic childbirth, mean the loss of a child in the perinatal period, the use of forceps or vacuum extractor, emergency caesarean section, labor pain, fear of epidural anesthesia, fear of labor, insufficient anesthesia, postpartum bleeding, post-hemorrhagic anemia, preterm labor, multiple pregnancy, severe toxicosis, a sense of loss of dignity or humiliating experiences for a woman during child delivery [5, 8, 17–19]. The risk group also includes patients who have experienced preterm labor, suffered from preeclampsia and hyperemesis of pregnant women [5, 8].

Six weeks after childbirth, the incidence of post-traumatic stress disorder ranges from 2.8 to 5.6%. After the process of traumatic childbirth, the prevalence of post-traumatic stress disorder associated with pregnancy and childbirth ranges from 3.1 to 15.9% in mothers from high-risk group. About 50% of women report having experienced a traumatic child delivery [8]. One of the first studies found that 75% of women within a few days after an emergency caesarean section considered it a traumatic event, 48% of women noted obsessive memories of child delivery, and 24% of women reported constant nervous tension [6]. Women who consider childbirth traumatic are more likely to meet the criteria for post-traumatic stress disorder [15]. This group of patients may not meet all the criteria for post-traumatic stress disorder, but it is possible to develop post-traumatic syndrome after childbirth, which significantly impairs their quality of life [15, 17].

Birth pain is one of the predisposing factors for the development of post-traumatic stress disorder, which emphasizes the importance of determining a woman's need for pain relief in time [19, 20]. Fear of labor pain and the process of childbirth can act as a factor causing the development of symptoms of PTSD associated with pregnancy and childbirth. Even with a successful completion of the labor, these

also contribute to a negative subjective assessment of the delivery, regardless of its objective characteristics [13, 15]. Fear of labor and labor pain increases women's requests for caesarean section and is an important and unique predictor for the development of post-traumatic stress disorder associated with pregnancy and childbirth [15, 17].

Thus, the fear of childbirth and a woman's experience of childbirth, both subjective and objective, can serve as a marker of the possible development of psychopathology in the postpartum period, which is important, in our opinion, to take into account in the clinical observation of such patients [15].

The risk factor is a subjective distress during child delivery, which includes the presence of negative emotions, loss of control over what is happening, the development of such a state as dissociation, characterized by a loss of expression of emotions, temporary loss of sense of reality [8, 17]. Lack of support during childbirth from medical staff or relatives reduces the woman's sense of control during childbirth, which increases the risk of postpartum mental disorders. Four weeks after childbirth, a woman's subjective perception of the delivery process is associated with post-traumatic syndrome, but eight weeks after childbirth this connection is less pronounced [15]. These indicators are associated with the fact that the subjective perception of childbirth by a woman is temporary, weakening over time from the moment of childbirth [15, 17]. Dissatisfaction with the delivery process also increases the risk of developing mental pathology in the postpartum period [17]. However, after childbirth, the importance of other factors that may affect the developing and continuing psychopathological symptoms increases. These may be difficulties with breastfeeding, difficulties in childcare, marriage problems, etc. [15, 17].

Prevention of post-traumatic stress disorder associated with pregnancy and childbirth is known to include a birth plan, when the woman is psychologically prepared for all stages of childbirth, the use of epidural anesthesia and skin-to-skin contact between the mother and the baby. The age of the mother of 35 years and older also contributes to this. The use of the Kristeller maneuver, instrumental delivery or delivery by caesarean section, ruptures of the perineum of degree III and IV, as well as the use of general anesthesia, manual separation of the placenta are risk factors for the development of post-traumatic stress disorder [19, 20].

Maternal assessment of the quality of communication with medical personnel of obstetric institutions is also a risk factor for the development of post-traumatic stress disorder associated with pregnancy and childbirth. These risk factors include lack of support from medical staff, lack of empathy, lack of information about their health and/or the child's health, negative communication between staff and patients [8, 19, 20]. For example, mothers whose children are in the intensive care unit often do not have a complete picture of the child's actual health status and believe that the child is healthier than s/he actually is [24]. Thus, in parents whose children are in the intensive care unit, factors related to the severity of the child's condition and the parent's perception of this condition are added to the main risk factors for post-traumatic stress disorder [24, 28]. Such cognitive and behavioral factors as negative cognitive perception of the childbirth process, the nature of memories of the psychotraumatic factor, avoidance behavior that consolidates the psychotraumatic event, contribute to the development of post-traumatic stress disorder associated with pregnancy and childbirth [9, 21].

Thus, in the process of childbirth, there is a large number of factors that can affect the development of post-traumatic stress disorder associated with pregnancy and childbirth, these ranging from fear of child delivery, objective and subjective characteristics of the delivery process itself to the woman's communication with medical staff, which, in our opinion, is important to take into account when working in obstetric institutions.

PRESENCE OF A PSYCHIATRIC HISTORY IN THE MOTHER AND A TRAUMATIC EVENT IN THE HISTORY. PERINATAL LOSS

There is evidence that psychiatric morbidity may be the leading cause of maternal mortality in the postpartum period [3]. An 80-fold increase in suicides has been estimated in women suffering from severe mental illness within a year after childbirth [3].

The risk factors for developing post-traumatic stress disorder associated with pregnancy and childbirth include: psychiatric history in the mother, anxiety and depression during pregnancy and after childbirth, unfavorable obstetric history, a traumatic event or several traumatic events experienced, perinatal loss, sexual abuse and the mother's specific personality features [5, 6, 8, 10, 13, 19, 20, 23].

Women who do not have other children experience more pronounced symptoms of grief and stress after perinatal loss [21]. Women with post-traumatic stress disorder associated with pregnancy and childbirth are at a five-fold risk of having a major depressive disorder and at a three-fold risk of generalized anxiety disorder [11].

Previous traumatic experience has been found to moderately influence the likelihood of developing post-traumatic stress in case of a new traumatic experience. Most often, additional traumatic events make it possible to predict the development of stress in certain population groups. Such predictors include exposure to multiple traumatic events, interpersonal trauma. The clinical prognosis tends to get worse when the woman is exposed to several stressful events within a short period of time [6].

Patients with confirmed or suspected personality disorders, such as borderline personality disorder, narcissistic personality disorder and dissocial personality disorder are at increased risk of developing a post-traumatic stress disorder, including the one associated with pregnancy and childbirth [6]. The presence of a neurotic disorder increases the risk of post-traumatic stress disorder emergence, with those associated with pregnancy and childbirth belonging there. It is possible to fully assess the impact of neurotic conditions on the development of post-traumatic stress disorder in the postpartum period only if exposure to a traumatic event has been identified in the process of direct clinical examination in the postpartum period [6].

Women suffering from affective disorders are at risk of relapse during pregnancy and after childbirth, especially if they do not take any medication [3, 36]. Mental disorders such as major depressive disorder, obsessive-compulsive disorder and post-traumatic stress disorder associated with pregnancy and childbirth have common risk factors, they are often comorbid with each other, but each of these diseases represents a separate nosology [15, 33, 34]. The frequent combination of postnatal depression and post-traumatic stress disorder makes it difficult to distinguish the unique characteristics of post-traumatic stress disorder associated with pregnancy and childbirth, such as traumatic intrusions and avoidance [15, 17].

During pregnancy the risk of recurrence of bipolar disorder (at least of one) is estimated to be 71%. The risk is doubled in women who have stopped taking mood stabilizers [3]. In patients with a major depressive episode, during pregnancy the frequency of recurrence was assessed to be 68% in the women who had stopped taking supportive medication, compared to 26% of women continuing to take the therapy [3]. It is very important to take supportive therapy during pregnancy, because in women with a history of bipolar affective disorder the frequency of postpartum psychoses can increase by 300 times, in women with a previous episode of postpartum psychosis, the frequency of relapse is more than 50% [3, 26].

The prevalence of post-traumatic stress disorder in the postpartum period significantly increases with stillbirth, the infant admission to the resuscitation or intensive care unit, the infant death in the intensive care unit, it accounts for 25 to 35% [8, 28]. In couples experiencing perinatal loss, an increased level of intensive stress in the man was noted to contribute to an increased stress level in the woman, and vice versa [4]. Perinatal loss is a traumatic experience that negatively affects not only the physical condition of patients, but also their mental state and social life [12]. According to a number of authors, in case of a perinatal loss, especially of an early one, parents are not given due attention, although the consequences of an early perinatal loss can become quite severe, as well as it happens in case of other severe traumatic events [12]. According to statistics, up to 25% of pregnancies result in perinatal loss, after which 50–80% of women repeat their pregnancy. However, such families do not receive sufficient specialist professional assistance. Up to 25% of women may have clinical manifestations of the post-traumatic stress disorder in the first month after their perinatal loss [12]. Symptoms of the post-traumatic stress disorder may occur in the subsequent pregnancy after a perinatal loss, and 4% of women develop a chronic post-traumatic stress disorder [12]. Women with symptoms of post-traumatic stress associated with pregnancy and childbirth are less likely to become pregnant again, they have a longer interval between pregnancies, their quality of life deteriorates in both the short and long term [7, 19, 20]. A year after the birth of a healthy child from a pregnancy after the perinatal loss, the prevalence of post-traumatic stress disorder associated with pregnancy and childbirth ranges between 4 to 6% [12]. Other factors aggravating a perinatal loss are: guilt and shame experienced, additional stress due to being in the intensive care unit, which is also supplemented by sleep disturbance and neuroendocrine changes, also by impaired relationship in the parental couple [8].

Often women who have suffered from a perinatal loss are not included into studies on post-traumatic stress disorder associated with pregnancy and childbirth, since the diagnosis of this condition can be confused with a pronounced component of loss [13].

Symptoms of post-traumatic stress disorder associated with pregnancy and childbirth are also observed in fathers actively involved into the follow up of their spouse's pregnancy, in particular, it concerns fathers who would see their child on an ultrasound scan [4].

The decrease in the symptoms of post-traumatic stress occurred faster in working women, as well as in men who did not drink alcohol, having a good education and income, as this enhanced their psychological protective mechanisms [4]. In contrast, the alcohol or psychoactive substances consumption increases the risk of post-traumatic stress disorder in men. In people using psychoactive substances, manifestations of post-traumatic stress disorder double as compared to the general population [4, 6]. The state of mental health deteriorates significantly in people simultaneously suffering from the post-traumatic stress disorder and beginning to use psychoactive substances [6, 31].

In a study focusing on the level of stress after a child loss, the stress level in women whose pregnancies resulted in a loss between the 22nd and 29th gestation weeks and after the 38th week was found to be higher than in those women who had a pregnancy loss between the 30th and 37th weeks.

It is believed that the increased level of stress in the loss of a child between the 22nd and 29th weeks is associated with unexpected pathological causes. Thus, premature birth is an important factor in the development of post-traumatic stress disorder associated with pregnancy and childbirth [4]. Mothers perceive premature birth as a threatening event, and often parents fear that the child may die [6]. After the 38th week of pregnancy with perinatal loss, the increased level of post-traumatic stress disorder is associated with the fact that at that point of gestation a woman is looking forward to seeing her child and is preparing for a normal outcome of pregnancy [4]. Also, the symptoms of post-traumatic stress disorder associated with pregnancy and childbirth were less pronounced in those men who saw their children after birth [4].

In a study of parents whose newborn had stayed in the intensive care unit, at the time of the baby's discharge, more than half of the fathers and over 60% of mothers reported that they had feared the child would die. The parents whose baby has survived feeling grief was similar to the response of parents who faced perinatal loss [6]. Of the 94 mothers whose babies had stayed in the intensive care unit, after their baby discharge 89 mothers reported that they used to have involuntary traumatic memories, the most common of which was experiencing the memory of their child possible death [6].

In some cases, parents did not give a name to their child fearing that he might not survive. Such manifestations at first can indirectly facilitate the emotional state of parents whose child is in the resuscitation unit, but it can also interfere with the development of child-parent attachment, with the development of an avoidance type of attachment [3, 6]. In some cases, mothers preferred not to visit the intensive care unit and not to see their baby [6]. During the first 24 hours after delivery 76% of mothers who have given birth to a term newborn experience feeling of love for their child, compared to 31% of those who have given birth to a preterm newborn. Half of the mothers of premature newborns had a 2-month delay in developing love for their child [6]. Both parents of a child with a very low body weight have a higher level of postpartum post-traumatic stress than the parents of a full-term infant [18].

Mothers whose child was born with a very low body weight have an increased risk of acute stress disorder (ASD), and subsyndromal levels of post-traumatic stress are equally high in both parents whose child was born with a very low body weight. The decreased number of mother's visits to the intensive care unit may result from her reaction to the traumatic event — the birth of a very low body weight baby [18]. Traumatic stress can limit parents' perception of information concerning the child's condition or the treatment measures [18]. According to the literature, after a perinatal loss, women have a higher risk of developing post-traumatic stress disorder associated with pregnancy and childbirth than men [4, 10]. From 18 to 78% of mothers whose baby was born prematurely are known to experience at least one symptom of PTSD [24].

Thus, a psychiatric history of a mother, as well as traumatic events in her life, tend to play an important part in the development of PTSD associated with pregnancy and childbirth. They appear to be its predictors. If a confirmed mental illness is already present, the patient's therapy should be under a strict control. This should be taken into account when working with these patients.

In our opinion, parents whose child was born premature and possibly with a somatic pathology, and staying in the resuscitation or intensive care unit should be separated into a special risk group. It should be considered by specialists in the perinatal departments who should develop measures to reduce the chances of post-traumatic stress disorder in this group of patients.

SOCIO-ECONOMIC FACTORS AND AVAILABILITY OF SOCIAL AND FAMILY SUPPORT

It is necessary to note a number of important socio-economic factors that can further increase the level of stress during pregnancy and after childbirth, they are: parents' young age, low income, low level of education, their unemployment/ no occupation, poor quality housing, living in disadvantaged areas [4, 8, 10, 17, 30].

In rare cases, in certain cultures birth of a child of a non-desired sex can aggravate manifestations of postpartum affective disorders [30].

One of the main risk factors and predictors of the development of post-traumatic stress disorder associated with pregnancy and childbirth is lack of social and family support. It includes problems in marriage, single mother/ pregnant woman, lack of a positive response to pregnancy from the father of the future child and close friends or relatives, lack of a health professional nearby who could answer questions of interest, chronic somatic diseases present during pregnancy [17, 30]. Moreover, the lack of social support is a stronger predictor of post-traumatic stress disorder than a previous traumatic event [6, 8]. Insufficient marital support, as well as insufficient social support, contributes to the increase of symptoms [23]. Social support can have a stress-buffering effect (it can reduce the influence of stress factors on a person) by suppressing stress reactions, such as an inflammatory response to stressors and weakening the activity of the sympathetic and hypothalamic-pituitary-adrenal axis. Women with insufficient social support are more likely to have concerns about childbirth and assess the child delivery process as negative [15].

RECOMMENDATIONS FOR MEDICAL STAFF OF OBSTETRIC INSTITUTIONS

It is important to clarify the medical history data concerning psychopathologically burdened heredity and presence of any psycho-traumatic factors and situations both during pregnancy and throughout the patient's life. Screening should be conducted to identify risk groups, since post-traumatic stress disorder associated with pregnancy and childbirth may not have been diagnosed or diagnosed incorrectly [8, 13, 35, 37]. This impairs the quality of patient's life, the functioning of the mother-child dyad and the well-being of the family as a whole [7, 13, 39, 40].

In order to detect post-traumatic stress disorder, medical staff of obstetric institutions should pay special attention to patients with a confirmed psychiatric illness, personality disorders included. It is essential to identify patients with behavioral disorders showing pronounced negative emotions. It is important to encourage manifestations of goodwill and positive emotions in parents experiencing stress due to the birth of a child and its condition [6].

In order to detect post-traumatic stress disorder during pregnancy and after childbirth, medical staff of obstetric institutions should closely monitor parents with low spirits, aggressive trends. It is possible to use special questionnaires used to detect classical post-traumatic stress disorder, as well as various modifications of these questionnaires for the period of pregnancy and the postpartum period [6].

A promising objective is to create new questionnaires specifically for post-traumatic stress disorder associated with pregnancy and childbirth. These questionnaires can be answered by the patients themselves or by a mental health specialist. It is also possible to adapt and validate some foreign questionnaires for this purpose [9].

If a pronounced mental health disorder of the mother is found out, it is necessary to consult a psychiatrist and, in rare cases, to take the mother to a psychiatric hospital [13]. The severity of clinical manifestations of post-traumatic stress disorder, as well as of other mental disorders during pregnancy and after childbirth can be perceived differently by the patient and the staff, so in each case an individual approach is necessary in assessing the severity of symptoms [14].

It has been shown that psychoeducation during pregnancy, cognitive psychotherapy, visiting groups teaching relaxation during childbirth, significantly reduce fear of childbirth and the number of caesarean section interventions [15, 25, 27]. One of the risk factors for post-traumatic stress disorder associated with pregnancy and childbirth is lack of a health professional who could answer questions concerning pregnancy, the infant's stay in the resuscitation or intensive care unit [17]. Thus, there is an urgent need to work out special measures diminishing anxiety in parents whose child is in the intensive care unit. There should be specially trained staff or an opportunity to communicate with the doctors more often, so that patients' questions could be promptly replied. [23]. The effectiveness of calm non-verbal music has been proven for parents suffering from stressful experience. It can also be used, against the background of the baby's stay in the resuscitation unit, in clinical practice [29].

With timely and quality medical care, including, availability to consult a psychiatrist, psychotherapist, the risks of development and manifestation of post-traumatic stress disorder associated with pregnancy and childbirth, as well as other mental disorders of the postpartum period becomes significantly reduced [17]. It is very important to study the mother's mental state, and it is also important to develop a successful progress of relations in the mother-child dyad [3].

Thus, post-traumatic stress disorder associated with pregnancy and childbirth is a unique type of classical post-traumatic stress disorder and this nosology requires a further detailed study, development of clear diagnostic criteria, competent differential diagnosis within the range of other mental disorders of the postpartum period, as well as the pregnancy period. In our opinion, it is necessary to involve specialists of different profiles for a more detailed study of post-traumatic stress disorder associated with pregnancy and childbirth in order to create preventive measures, as well as to develop a plan for referring patients to specialist staff if a psychopathology was revealed during pregnancy and after childbirth, since the well-being of the mother-child dyad, the well-being of the family and further well-being of future generations depend on competent and timely medical care.

ADDITIONAL INFORMATION

Author contribution. Thereby, all authors made a substantial contribution to the conception of the study, acquisition, analysis, interpretation of data for the work, drafting and revising the article, final approval of the version to be published and agree to be accountable for all aspects of the study.

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PATHOPHYSIOLOGICAL FEATURES OF GLIAL CELL CHANGES AND MARKERS OF BRAIN TISSUES DAMAGE IN TBI

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Abstract. Traumatic brain injury (TBI) is the leading cause of mortality and psychiatric disorders among neurologic pathology. In many patients, TBI leaves long-term sequelae that may involve both mild cognitive impairment and severe disability. It is known that the mechanisms of damage in traumatic brain injury can be primary, related to the mechanical impact on the brain, and secondary, mainly caused by astrocytes, microglia and infiltrated immune cells from peripheral tissues that lead to neuronal and vascular dysfunction. Because these mechanisms, particularly secondary injury, remain incompletely understood, there are difficulties associated with the diagnosis and treatment of TBI. In search of a solution to this problem, substantial data on the quantification of biomarkers of traumatic brain injury have accumulated in recent decades, which may provide a clinically accessible window to study the mechanisms, diagnosis, monitoring, and prediction of brain injury outcomes. The article is a brief review of posttraumatic changes in brain tissue associated with ionic disturbances, activation of astro- and microglia, involvement of immune system cells, and major biomarkers of brain injury isolated from blood and cerebrospinal fluid.

Key words: traumatic brain injury; astroglia; microglia; damage; biomarkers.

ПАТОФИЗИОЛОГИЧЕСКИЕ ОСОБЕННОСТИ ИЗМЕНЕНИЯ ГЛИАЛЬНЫХ КЛЕТОК И МАРКЕРЫ ПОВРЕЖДЕНИЯ ТКАНЕЙ МОЗГА ПРИ ЧЕРЕПНО-МОЗГОВОЙ ТРАВМЕ

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Резюме. Черепно-мозговая травма (ЧМТ) является основной причиной смертности и психических расстройств среди неврологической патологии. У многих пациентов ЧМТ оставляет долгосрочные последствия, которые могут

быть связаны как с легкими нарушениями когнитивных функций, так и с тяжелой инвалидизацией. Известно, что механизмы повреждения при ЧМТ могут быть первичными, связанными с механическим воздействием на головной мозг, и вторичными, в основном вызванными астроцитами, микроглией и инфильтрированными иммунными клетками из периферических тканей, которые приводят к нейрональной и сосудистой дисфункции. Ввиду того, что эти механизмы, в частности вторичное повреждение, остаются не до конца изученными, существуют сложности, связанные с диагностикой и лечением ЧМТ. В поисках решения этой проблемы в последние десятилетия накопились существенные данные о количественной оценке биомаркеров ЧМТ, что может обеспечить клинически доступное окно для изучения механизмов, диагностики, мониторинга и прогнозирования исходов травмы головного мозга. Представлен краткий обзор посттравматических изменений в ткани головного мозга, связанных с ионными нарушениями, активацией астро- и микроглии, участием клеток иммунной системы, а также основных биомаркеров повреждения головного мозга, выделенных из крови и цереброспинальной жидкости.

Ключевые слова: черепно-мозговая травма; астроглия; микроглия; повреждение; биомаркеры.

Traumatic brain injury (TBI) is a global problem of public health service in the modern world. Traumatic injuries of the brain are the most relevant forms of neurologic pathology [7]. Epidemiological studies identify a stable increase of the number of traumatic brain injuries, particularly in big cities [6]. The prevalence of traumatic brain injury in the Russian Federation is 130–400 cases per 100 thousand population [9]. The risk of head injury has increased taking into account new dynamics of modern technological society development. Car accidents, extreme sports, and armed conflicts have increased the frequency of TBI [6].

According to the World Health Organization TBI is annually diagnosed in over 10 million patients worldwide within the period of 5 years, and 200–300 thousand people die. The principal causes of the population disabilities following TBI are considered mental and cognitive disorders, severe motor and speech impairments, posttraumatic epilepsy, etc. A stable rise of disability following TBI in able-bodied population (mean age 20–40 years old) has increased [13, 14]. Due to this fact there is a negative increase in realization of labour potential of the country (budget loss of about 495 milliard roubles a year), but at the same time huge resources are spent to provide medical institutions with everything required for the treatment and rehabilitation of disabled people [1, 12].

Disability in case of traumatic brain injury is caused by both primary involvement of the brain, and development of new clinical syndromes of dysregulation mechanisms and decreased adaptable reserves after some time period and during the period of complications [3].

This paper presents a review of relevant studies aimed at understanding the problems connected with TBI diagnosis. The paper gives an analysis of such problems as damage of the brain cells in case of TBI, participation of microglia and astroglia in TBI pathogenesis, , as well as the issue connected with the study of TBI biomarkers.

ETIOLOGY AND PATHOPHYSIOLOGY OF TRAUMATIC BRAIN INJURY

TBI results from intense collision, acceleration-delay and rotary motion of the brain that leads to its functioning disorder. It is possible to make pathophysiological differentiation between primary and secondary brain damage. Primary brain damage can be caused by: a) direct action of mechanical force leading to local damage, characterized by fractures, cerebral hemorrhage, and local neuron necrosis, or b) fast accelerating and slowing forces which determine stretching of the brain tissue with associated diffuse axon damage mainly manifested on the level of brainstem and callous body that can remain there within several months after the trauma. The secondary brain damage caused by biochemical and cellular changes, which are secondary to primary damage, is connected with numerous factors, including lipid peroxidation, mitochondrial dysfunction, oxidative stress, excitotoxicity, neuroinflammation and axonal degeneration.

DAMAGE OF THE BRAIN CELLS IN CASE OF TRAUMATIC BRAIN INJURY

Direct mechanical exposure leads to immediate appearance of irreversible mechanical damages of the skull bones, its coats, the brain vessels and tissues,differing in severity [2]. In case of primary damage there is a disorder in the structure of neurons and glial cells, followed by axon synaptic ruptures or distensions formation, damage of blood-brain barrier, vascular thrombosis appearance, and the integrity of vascular wall is disturbed [47].

After the trauma a perifocal zone is formed around the centre of the primary damage where the cells remain viable [16], but become extremely sensitive to the mildest changes of oxygen and nutrition transportation (penumbra zone) [8].

Due to considerable oxygen and glucose demand by the brain tissue there is a displacement of perfusion in the area of damage that leads to lack of substrates and accumulation of toxic metabolites. The speed of energy production by brain cells is changed which means failure of ionic gradients, decreased membrane potentials.

The involved cells release glutamate from intracellular reserves [25, 53]. Glutamate causes death of nervous cells by some mechanisms. It initiates both types of glutamate receptors, NMDA (*N*-methyl-D-aspartic acid) and AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) that leads to Na^+ afflux, K^+ efflux, and intensive Ca^{2+} afflux to neurons [29]. This process is called excitotoxicity. It leads to uncontrollable and stable increase of cytosolic calcium that causes disorders in mitochondrial transportation of electrons and stimulates the functioning of many calcium-dependent enzymes, including lipases, phospholipases, calpains, nitrogen oxide synthase, protein phosphatase and different protein kinases [15].

In case of prolonged lack of energy neurocytes and glial cells are depolarized, being changed functionally and structurally [34]. Damage and energy deficiency of cells in tissue leads to disorders in their interaction, change of intercellular fluid components, release of proinflammatory agents that causes glia activation.

ROLE OF MICROGLIA IN NEUROINFLAMMATION

Microglia is a subtype of CNS glial cells that have the function of resident macrophages [36]. They usually utilize the accumulated metabolism products and also influence the processes of training and memory, regulating destruction of cells and neurogenesis.

Similar to peripheral cells, microglia releases pathogen recognition receptors, such as toll-like receptors (TLR) and NOD-like receptors (NLR), and, therefore, reacts to pathogen-associated molecular patterns (PAMP), and endogenously produced damage-associated molecular patterns (DAMP) that are secreted by broken neurons and other CNS cells [32]. They also express receptors of some other factors that are released by broken neurons, including adenosine triphosphoric acid, glutamate, growth factors and cytokines. Microglia is composed by antigen-presenting cells and interacts with T-lymphocytes and activates markers of cellular surface, such as MHC II and CD86, as well as molecules of adhesion and complement receptors [32].

There are known to be two phenotypes of activated microglia: pro-inflammatory M1 and anti-inflammatory M2. Activation according to M1 type leads to the synthesis of tumor necrosis factor α (TNF α), interleukins (IL)-12, -6, -1 β, NO, oxygen active forms (OAF), chemokines CCL2, CXCL9, CXCL10 [23]. M2-microglia produces anti-inflammatory IL-4, IL-13 that have neuroprotective action [52]. However, it is obvious that highly reactive condition of activated microglia according to M1 type in response to DAMP and other extracellular signals of damage leads to release of high levels of proinflammatory and cytotoxic mediators that promote the dysfunctions of neurons and death of cells [24, 38]. After TBI activated microglia quickly migrates to the zone of damage and creates a barrier between involved and healthy tissue and stimulates phagocytosis in the involved tissue that, consequently, is a positive side of microglia participation.

For example, after an experimental study of fluid-percussion TBI model in rats Iba-1 labelling demonstrates that microglia is hypertrophic and gets amoebiform in the cerebral cortex and thalamus that remains within 7 and 28 days after TBI and correlates with subacute and chronic course in experimental TBI model [22].

ROLE OF ASTROGLIA IN NEUROINFLAMMATION

Astrocytes of the brain are subdivided into fibrillar, located mainly in the brain white substance, and protoplasmic, located in the brain grey substance. Damage of these cells leads to disturbance of their basic functions: those of neuron energy supply, synaptogenesis, transportation of neurotransmitters, normal ionic balance maintenance, formation of blood-brain barrier (BBB).

In response to mild or moderate tissue damage astrocytes are exposed to hypertrophic reactive astrogliosis that includes molecular, structural and functional changes. Severe tissue damage causes degeneration of nervous and glial cells, destruction of vessels and intense immune answer that leads to formation of tissue compartments with various forms of reactive astrogliosis. Directly near the damage astrocytes proliferate and intertwine, forming astroglial cicatrix that surrounds and limits distribution of intensive inflammatory reaction in the damaged area [20].

Under the influence of DAMP, ionic changes and deficiency of energy there is transformation of cells to reactive astrocytes, and the amount of them increases in the damaged area after the trauma. Patients with TBI and experimental TBI mice had increased expression of endothelin-1 (ET-1) [42], and ET-1 increase promoted transformation to reactive astrocytes through ETB receptor in mice with fluid-percussion TBI model [42]. Some inflammatory cytokines and chemokines also cause astrogliosis. IL-1 provides transformation of astrocytes to the reactive form [30], at the same time IL-1 receptor antagonist reduces astrogliosis of hippocampus in such experimental TBI model, as controlled cortical damage [50].

Damaged neurons release high mobility group protein B1 (HMGB1) that induces IL-6 secretion by microglia cells, IL-6 activates the water channel of astrocyte aquaporine-4

(AQP4), participating in water absorption [39]. The negative side of reactive astrocytes is that they can directly increase intracranial pressure because of cytotoxic hypostasis and develop harmful mediators of inflammation which aggravate brain damage.

Activated astrocytes can also release matrix metalloproteinase-9 in response to mechanical pressure [48]. As a result of its activation intercellular contacts that influence the increase of penetration of blood neutrophiles, leukocytes and monocytes into the trauma center that leads to the increase of BBB permeability and, as a consequence, to edema aggravation.

To protect the neurons astrocytes produce soluble factors, such as transforming growth factor β (TGF-β) and prostaglandins which can inhibit microglia activation [37], as well as provide with nutrients and support homeostasis of extracellular liquid due to glutamate and potassium absorption increase [33].

According to the information above it could be said that glial cells can render various effects, both negative and positive that, in turn, will be reflected in restoration of neuron functions and plasticity during tissue reorganization. At the beginning glia activation has a protective character, delimiting the damage area and supporting viability of damaged neurons, stimulating neurogenesis, but subsequent prolonged release of proinflammatory cytokines and formation of glial cicatrix causes brain disorders. To understand these activities better, further studies of their participation in TBI pathogenesis are necessary.

By the present moment a considerable number of biomarkers that can indicate damages of neurons and neuroglia have been studied. The scientific community actively conducts researches on identification of the biomarkers unambiguously characterizing TBI which could be included further in its diagnostic criteria.

Search of these biomarkers is important to predict possible complications and to use them as indicators for assessing the severity of TBI in patients.

LIQUID BIOMARKERS

1. *Neurofilament light polypeptide (NfL),* released from damaged axons, was suggested as a viable biomarker of mild TBI [31, 51, 59].

2. *Soluble vascular adhesion protein 1 (sVAP-1)* is increased in plasma correspondingly with TBI severity [41]. The threshold value was 8,61 nmol/ml per hour, and those patients who had higher levels, presented 25% death rate increase.

3. *Galectin-3,* a member of lectins family, participating in microglia activation, has increased concentration in plasma in TBI patients, and is also an indicator of hospital mortality [46].

4. *High mobility group box 1 protein* (HMGB1) is translocated from nucleus to cytoplasm at the beginning of TBI, later it penetrates phagocytic microglia and represents cytokine and inflammation marker which is a predictor of one-year death in patients with TBI [55, 56], like an increased level of *copeptin hormone* [26].

5. *S100B* is an endocellular calcium-binding protein found in astrocytes, which is one of the most widely studied TBI biomarkers [17]. It has been demonstrated that serum S100B concentration during an acute phase of TBI negatively correlates with brain communication at rest condition that is identified by functional MRI [54]. One study has shown that addition of S100B test to the clinical guidelines on TBI management can become economically effective and lower the frequency of CT [21]. It has been shown that serum S100B concentration is considerably changing in the course of time that is significant for early prognosis [28]. It is interesting that the patients who had surgeries due to their fractures of backbone or lower extremities, demonstrated essential increase of blood S100B concentration compared with presurgical concentration [57]. It has also been shown that the placement of external ventricular drainage influences the levels of S100B though this time S100B cerebrospinal fluid and serum levels above 0.7 mkg/dl correlate with 100% death in case of TBI and subarachnoid hemorrhage [35].

It has been identified that S100B levels vary depending on type and number of TBI lesions. S100B test can be used in a group of patients with mild TBI with alcoholic intoxication. S100B test was more accurate in sober patients compared with alcohol intoxication patients. Serum S100B 24-hour levels can be used as a screening tool for early identification of patients with brain death risk following severe TBI. S100B can be an effective tool of TBI treatment monitoring, and one study has shown that S100B levels decrease after hyperosmolar therapy. It has been supposed that S100B samples taken within 12 hours after traumatic damage, have smaller prognostic value compared with S100B samples taken 12–36 hours after the trauma. It was also suggested that urine S100B levels had prognostic value compared with serum S100B levels. It has been shown that the combination of S100B levels with glial fibrillary acidic protein levels (GFAP) leads to precise prognosis of one-year death following TBI.

6. *Tau-protein* (Microtubule-associated protein tau, MAPT) is a protein that has its role in neuron development, axon stabilization and neuron polarity. It has been noticed that serum and cerebrospinal fluid tau-protein levels can be considered as TBI biomarker, because pathologicoanato-

mic examination demonstrated the increased levels of tau-protein even if macroscopically visible damages were not noticed, this means that some damages nevertheless could occur [45].

It has also been shown that the levels of the split serum tau-protein are significantly higher in case of severe TBI compared with control group [49]. The levels of total tau correlated positively with clinical and radiological TBI indicators [18]. Poor outcomes in case of severe TBI were identified in patients with higher level of blood serum tau-protein [40].

7. *Neuron-specific enolase (NSE)* increases similarly to S100B in TBI patients [44], and it is progressive depending on the trauma severity [60]. Medicine treatment (memantine) of patients with moderate TBI leads to significant decrease of blood serum NSE level and to the improvement of indicators according to Glasgow coma scale [43]. However, some foreign authors consider that NSE can be not so accurate or clinically useful compared with S100B [54]. But, again compared with S100B, the increase of NSE has been more closely connected with brain death prognosis after severe TBI [19].

8. *Nesfatin-1* is connected with inflammation and is an independent predictor of hospital mortality. Its concentration in plasma is connected with TBI severity, and it can become a reliable prognostic marker of these traumas [58].

9. *Resistin*, also called adipocyte-specific secretory factor (ADSF), is a secretory factor specific to adipose tissue. Plasma resistin levels increase from the 6th hour after the trauma, and reach its peak in 24 hours [27]. The study demonstrated that resistin is an independent predictor of one-month death of patients.

Experimental and clinical studies have shown that in case of TBI microglia cells and astrocytes more frequently produce such cytokines as [4]:

- *Interleukin-1 β* (IL-1 β) is an anti-inflammatory cytokine stimulating apoptosis and phagocytosis of the cells, causing fever. Active IL-1 β secretion after TBI contributes to the increase of excitability and excitotoxicity by glutamatergic and gamma-aminobutyric acid-ergic mechanisms and to change of concentration of calcium ions that can potentially lead to epilepsy development. Increased correlation of IL-1 β in liquor and blood serum during TBI acute phase is connected with an increased risk of posttraumatic epilepsy development [11]. Thus, IL-1 β plays a significant role in inflammatory processes in case of TBI and can be a marker of TBI severity and risk of posttraumatic epilepsy development.
- *Interleukin-6* (IL-6) is characterized by both proinflammatory, and anti-inflammatory features. IL-6 is considered to be the basic regulator of inflammatory responses which provides short-term protection against infectious process and tissue damage, has neuroprotective function. The role of IL-6 in case of TBI has been investigated in some clinical studies [5]:
  - The increase IL-6 in the cerebrospinal fluid of the ventricles patients with TBI, and also communication between IL-6 and production of the factor of neuronal growth was noticed that has allowed to assume considerable intracranial production IL-6 after TBI;
  - Blood IL-6 level increase within 48 hours after severe TBI is connected with unfavourable delayed clinical outcomes;
  - The analysis of serum IL-6 levels in patients with severe TBI has demonstrated that the highest IL-6 concentration has been found on the first day of hospitalization and was associated with the formation of multiple organ disfunction, sepsis and adverse neurologic outcome;
  - Liquor and blood IL-6 level reaches its peak in 24–28 hours after TBI;
  - IL-6 blood plasma concentration is increased by the time of making the diagnosis of the brain death.

Thus, high levels of IL-6 measured in blood and liquor, are associated with poor outcomes of trauma and higher risk of death outcome, being a possible predictor of intracranial hypertension after isolated TBI.

- *Tumor necrosis factor α (TNF α)* is involved in pathophysiological processes in case of many diseases and conditions, particularly systemic inflammatory response syndrome (SIRS), combined trauma, massive burns and rheumatoid arthritis [10]. The experimental studies have identified that:
  - TNF α activity is increased during the first hours after TBI and it is not found in blood serum on 3–7 days after the trauma;
  - Due to the influence of IL-1 on astrocytes and microglia there occurs the production of proinflammatory cytokines, including TNF α which will also stimulate IL-6 production by glial cells;
  - There is lack of studies describing the role of TNF α in TBI pathogenesis; TNF α concentration reaches its peak during the first hours after TBI and correlates with high mortality and formation of multiple organ disfunction;
  - Correlation of TNF α level and development of intracranial hypertension is noted.

In general, cytokines, belonging to the group of hormone-like proteins and peptides, which activation, according to the opinion of both Russian and foreign scientists, leads to various phenomena which can be observed in the brain after TBI, for example pyrexia, neutrophilia, edema,

disorders in BBB permeability, that have an important role in intercellular communications and stimulate reparative processes, such as gliosis.

However, gliosis, in its turn, causes further production of cytokines by hypertrophied astrocytes and microglia cells, in addition to mediators secreted by cells of peripheral immune system: polymorphonuclear leukocytes which penetrate through the weakened BBB that can lead to further brain damage.

Despite a considerable number of biomarkers known nowadays that can be associated with damages in case of TBI, most part of them are not highly specific and can be characteristic for other pathologies. Thus, among the above described biomarkers it is possible to allocate a number of the most perspective for diagnosis and assessment of dynamics in patients with TBI. They are neurofilament light polypeptide, neuron-specific enolase and glial fibrillary acidic protein. So, for example, GFAP was included in TBI diagnostic criteria by U.S. Food and Drug Administration [61].

CONCLUSION

TBI still remains one of the most severe types of trauma, even in case of mild TBI patients are likely to have prolonged disorder of cognitive functions that can be connected with a prolonged neuroinflammation course which types differ in pathology and outcome.

Detailed studies and evidences are needed to have a clear idea about the damage and regeneration of nerves. The available data do not make it possible to specify a definite role of inflammation after TBI due to its complex molecular and cellular interactions. The studies demonstrate that the mechanisms provoked by the trauma, have a protective function during acute inflammation and influence negatively in long-term prospect. The basic participants starting the cascade of these responses, are astrocytes and microglia, that is why biologically active factors and functional molecules, formed by them are, therefore, likely to be attractive targets for studying.

Pathophysiological markers of the brain tissue damage give evidence to a variety and mosaic structure changes in case of different types of trauma that underlines importance of continuation of TBI and its markers study. Allocation of the spectrum of the basic biomarkers — TBI indicators of different severity levels — could help to simplify diagnostics and further control of patients after trauma.

Thus, future studies of neuroinflammation mechanisms will allow to develop new algorithms of treatment, able to limit influence of secondary brain damage and to improve a long-term prognosis for patients.

ADDITIONAL INFORMATION

Author contribution. Thereby, all authors made a substantial contribution to the conception of the study, acquisition, analysis, interpretation of data for the work, drafting and revising the article, final approval of the version to be published and agree to be accountable for all aspects of the study.

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CHAPEROME: HISTORICAL PERSPECTIVE AND CURRENT CONCEPTS

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Abstract. The life cycle of cells is accompanied by constant synthesis, transport and degradation of polypeptide chains — proteins and signal sequences. Each polypeptide chain has four levels of structure, and its adoption of the correct spatial conformation is necessary for the expression of the function of the molecule. Hydrophobic interactions or the formation of sulfide bridges can prevent the formation of the correct conformation. Moreover, the high-order structures of proteins are disrupted by various stress responses to the cell. In the course of studying the processes of protein synthesis and aggregation, specific highly conserved proteins were identified that can bind to a newly synthesized or damaged polypeptide, imparting a functional structure due to the sequential connection with recognition domains. These proteins are called molecular chaperones. This includes the superfamily of heat shock proteins, the synthesis of which is a nonspecific cell response to stress. To study the processes of proteostasis, it is necessary to understand that these proteins act only in close relationship with cochaperones and other auxiliary molecules. Such aggregates are called chaperomes, or chaperone machineries, and are of considerable interest in biomedical research. This review discusses the historic perspective for chaperones and chaperome as a supramolecular complex as well as their place in cell proliferation.

Key words: molecular chaperones; heat shock proteins; chaperome.

ШАПЕРОМ: ИСТОРИЧЕСКАЯ ПЕРСПЕКТИВА И СОВРЕМЕННЫЕ ПРЕДСТАВЛЕНИЯ

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Резюме. Жизненный цикл клеток сопровождается постоянным синтезом, транспортом и деградацией полипептидных цепей — белков и сигнальных последовательностей. Каждая полипептидная цепь обладает четырьмя уровнями структуры, и принятие ею правильной пространственной конформации необходимо для экспрессии функции молекулы. Препятствовать формированию правильной конформации могут гидрофобные взаимодействия или образование сульфидных мостиков. Более того, структуры высокого порядка белков нарушаются при различных стрессовых ответах на клетку. В ходе исследования процессов синтеза и агрегации белков были выявлены особые консервативные протеины, способные связываться с новосинтезированным или поврежденным полипептидом, придавая за счет последовательной связи с доменами узнавания функциональную структуру. Именно эти белки назвали молекулярными шаперонами. В их число входит суперсемейство белков теплового шока, синтез которых является неспецифичным ответом клетки на стресс. Для изучения процессов протеостаза необходимо понимание, что данные белки действуют лишь в тесной взаимосвязи с кошаперонами и другими вспомогательными молекулами. Такие совокупности называются шаперомом, или шаперонной машиной, и они представляют значительный интерес в биомедицинских исследованиях. В данном обзоре литературы представлены основные исторические этапы понимания шаперонов и шаперома как супрамолекулярного комплекса и их место в жизнедеятельности клетки.

Ключевые слова: молекулярные шапероны; белки теплового шока; шапером.

Thanks to the research of the early twentieth century, it became clear that many polypeptides (typically small single-domain proteins) can easily restore their native structure by themselves in vitro, while others (more complex, multi-domain or oligomeric proteins) adopt the necessary topology only in the presence of additional molecules that are not part of the polypeptide of the final native protein. [22]. These molecules were identified as proteins and were named molecular chaperones. The term “molecular chaperone” was first used in 1968 in order to describe the role of nucleoplasmin in the assembly of DNA and histones into nucleosomes [11]. The name arose from the fact that nucleoplasmin promotes histone-histone interactions with the formation of a correct oligomeric form, preventing aggregation. It does this without forming a part of the nucleosome by itself or defining the modification of the nucleosome. Therefore, nucleoplasmin assumes the role of a chaperone.

Later, the term “molecular chaperone” was expanded to include a widespread chloroplast protein called the RuBiSCo (ribulose bisphosphate carboxylase) large subunit binding protein now known as chloroplast chaperonin, which prevents the formation of insoluble precipitate by newly synthesized large rubisco subunits. These large subunits are known to be prone to improper assembly not because of electrostatic interactions, but because they expose highly hydrophobic surfaces to the aquatic environment. Although early experiments did not determine whether chaperonin promotes folding or assembly, more recent work with mitochondrial chaperonin has proven that this protein functions at the stage of folding [15, 29]. For some time, the term “molecular chaperone” was limited to two proteins; its modern use began with the assumption that its meaning should be expanded to describe the function of a larger group of proteins that were supposed to promote folding and assembly reactions in various cellular processes [9, 17]. Since the 1990s, this definition has been constantly being refined to take into account other discoveries concerning the role of chaperones in the processes of cell proteostasis.

The role of molecular chaperones in the folding, assembly, and intracellular translocation of proteins is a constant subject of research. The following conclusions are valid for normally functioning cells.

Chaperones of organelles such as EPR (endoplasmic reticulum) play a key role in the folding of newly synthesized proteins [16, 23]. Mitochondrial membranes have an isolated population of chaperones responsible for correcting or disassembling proteins damaged during cellular respiration [40].

Cytosolic chaperones play a key role in the folding, transport, and biological activity of a number of proteins used for transport to specific organelles, such as the nucleus and mitochondria [7, 28, 42].

Nuclear chaperones, unlike others, bind to proteins after folding due to ionic forces, and play a supportive role in the structural organization of macromolecular chromatin complexes [30].

Membrane-bound chaperones, found only on cells of solid and hematological tumors, are involved in the proliferation, migration, and immunogenicity of cancer cells [4, 38].

The list of detected chaperones is constantly updated. Enzyme-like cofactors protein disulfide isomerase and peptidyl-prolyl cis-trans-isomerase, which catalyze trans- to cis-proline isomerization and are usually considered as EPR chaperones, were only discovered in 1992 [37]. The chaperone family included both prokaryotic and eukaryotic proteins of different structures and localization.

However, differences in names and the lack of a clear division into families significantly complicated the studies of chaperones. A significant part of human chaperones began to be identified as human heat shock proteins (HSP), stress-sensitive proteins necessary to combat heat and other protein-toxic stresses. Soon after that, in 2003–2005, it became clear that constitutively expressed members such as Hsc70 (HSPA8) in the HSP70 family can also be encoded within the HSP family. This is how the division into stress-induced and constitutive chaperones appeared [18].

With the development of protein crystallography and genetic analysis, the first attempts to improve the classification were made — there appeared a division into families (by molecular weight), within which specific representatives were identified (by coding genes). However, even after analysis of the human genome, the names used for human chaperones in the literature were rather chaotic: up to ten different names could be found for the same gene product. Moreover, almost identical names were used to refer to different gene products. For example, HSPA1B was named HSP70–2, whereas HSP70.2 refers to the member HSPA2, which is specific for testicles. The first steps in dividing chaperones by genes and functions at the same time were only made by 2000.

The revolutionary work of Professor Harold Kampinga was particularly successful in shaping the classification [20]. The proposed nomenclature was based on the encoding assigned by the HUGO Gene Nomenclature Committee and used in the National Center of Biotechnology Information Entrez Gene database for the heat shock genes. In addition to this nomenclature, a list of the human Entrez gene identifiers and the corresponding Entrez gene identifiers for the mouse orthologs was provided. In this work, tables were presented for each superfamily of human chaperones (Fig. 1).

Chaperones seem to act sequentially in protein folding pathways, binding to intermediates that are at various stages of topology formation, and then transferring them to the next chaperone or chaperone complex in the cascade, eventually releasing the competence native protein. Binding usually involves the interaction of chaperones with hydrophobic residues on the surface of unfolded proteins, and

	Gene name	Protein name	Old names	Human gene ID	Mouse ortholog ID
HSP A					
1	*HSPA1A*	HSPA1A	HSP70-1; HSP72; HSPA1	3303	193740
2	*HSPA1B*	HSPA1B	HSP70-2	3304	15511
3	*HSPA1L*	HSPA1L	hum70t; hum70t; Hsp-hom	3305	15482
4	*HSPA2*	HSPA2	Heat-shock 70kD protein-2	3306	15512
5	*HSPA5*	HSPA5	BIP; GRP78; MIF2	3309	14828
6	*HSPA6*	HSPA6	Heat shock 70kD protein 6 (HSP70B′)	3310	X
7	*HSPA7*[a]	HSPA7	Heat shock 70kD protein 7	3311	X
8	*HSPA8*	HSPA8	HSC70; HSC71; HSP71; HSP73	3312	15481
9	*HSPA9*	HSPA9	GRP75; HSPA9B; MOT; MOT2; PBP74; mot-2	3313	15526
10	*HSPA12A*	HSPA12A	FLJ13874; KIAA0417	259217	73442
11	*HSPA12B*	HSPA12B	RP23-32L15.1; 2700081N06Rik	116835	72630
12	*HSPA13*[b]	HSPA13	Stch	6782	110920
13	*HSPA14*	HSPA14	HSP70-4; HSP70L1; MGC131990	51182	50497
HSP H					
1	*HSPH1*	HSPH1	HSP105	10808	15505
2	*HSPH2*[b]	HSPH2	HSPA4; APG-2; HSP110	3308	15525
3	*HSPH3*[b]	HSPH3	HSPA4L; APG-1	22824	18415
4	*HSPH4*[b]	HSPH4	HYOU1/Grp170; ORP150; HSP12A	10525	12282

Fig. 1. HSP70 family classification [20]
Рис. 1. Классификация семейства HSP70 [20]

release often involves the hydrolysis of ATP. The formation of functional complexes is not related to certain consensus amino acid sequences in the substrate protein, but rather is determined by the location of hydrophobic residues and conserved recognition sites [14, 43]. The researchers understood that a single chaperone would not provide stable work to maintain proteostasis. That is why, at the turn of the century, the identification of various adapter proteins, transport proteins, and signaling molecules in combination with chaperones also began.

The term "chaperome" was introduced in 2006 to denote a combination of chaperones, co-chaperones, and related factors [41]. The initial list of the human chaperome was published in 2013, and it reported 147 bioinformatically predicted members [13]. It included members of heat shock protein 90 (HSP90), HSP70, HSP60, HSP110, HSP40 (also known as DNAJ proteins), HSP10 and small HSP (sHsp), as well as their co-chaperones and participants of folding, the enzymes peptidyl-prolyl-isomerase (PPI) and protein disulfide isomerase. The name of each HSP family comes from the molecular weight of the original founding member and follows the current nomenclature. In eukaryotes, most families also include components specific to organelles, such as those expressed in the endoplasmic reticulum and mitochondria. Later studies expanded the list to 332 chaperones and co-chaperones, represented by 88 chaperones (27%), 50 of which were ATP-dependent, and 244 co-chaperones (73%) [2, 3]. Several proteins containing tetratricopeptide repeats (TPR) domain were also included based on their functional interactions with selected chaperones.

Analysis of protein expression in immortalized human cells (both in untransformed and cancer cells) identified chaperome components as some of the most common proteins in these cells [36]. HSP90 were the most common ones, accounting for an average of 2.8% and, together with HSP70, up to 5.5% of the total protein mass. Referring to the aforementioned database of 147 chaperome members, these proteins together account for 7.6% of the total number of polypeptides and 10.3% of the total protein mass in human cervical cancer cells HeLa. The chaperones HSP60 and HSP110 accounted for another 3.3% of the total protein mass, and 1.5% of the total mass consisted mainly of regulatory co-chaperone proteins for HSP90 and HSP70. More specifically, isoforms HSP90AA1 and HSP90AB1 (HSP90α and HSP90β) and two HSP70 proteins, constitutive HSPA8 (heat shock 70-related, HSC70) and heat shock-induced HSPA1A/B proteins represented the vast majority of chaperones of the corresponding families. In addition, all known HSP90 co-chaperones were substoichiometric with respect to HSP90. For example, the ratio of co-chaperone to HSP90 was 1:34 for AHA1, an activator of HSP90 ATPase activity, 1:46 for kinase-binding CDC37 [26], and 1:16 for HOP (HSP70-HSP90 adapter protein, also called STIP1, which binds HSP90 to HSP70) [5, 34]. Similarly, the ratio of co-chaperones to HSP70 was 1:5.5 for various J-domain co-chaperones that activate HSP70 to certain functions, and 1:7 for HSP110, which act as nucleotide exchange factors for HSP70 [10, 31].

These chaperones and co-chaperones are organized as interacting protein networks (Fig. 2). In eukaryotes, there are distinct and independent networks of chaperomes, with the main chaperone, such as HSP90 or HSP70, functioning with the help of a number of co-chaperones, each of which has a specific set of functions necessary for cell proteostasis. Not only RNA and ribosomes are involved in the synthesis of each protein, but also chaperome complexes characterized by close interconnection that can give the newly synthesized protein the topology required to perform its functions, and then send it to the appropriate cell compartment.

The chaperome is an extremely dynamic structure: HSP representatives included in it, the strength of bonds between proteins, and the functions performed depend on the state of the cell and its microenvironment. For example, studies of yeast cells have shown that Hsp82 is able to form stable networks with other chaperones during heat shock induction [19]. The accumulation of damaged proteins activates the expression of HSP110, which are able to "direct" HSP to the function of protecting and refolding protein aggregates [12]. Moreover, it has been found that constitutive and stress-induced forms of HSP70 and HSP90 are capable of forming functional oligomers in response to toxins or nutrient elimination [1, 27]. Thus, various manifestations of cellular stress can change the strength of interaction between both chaperone members and individual complexes. This reorganization of a higher order than the chaperone-substrate can lead to the emergence of new functions that are not normally expressed, but which may be required to counteract stress factors. It is worth noting that the same feature can maintain cell viability in case of a pathology.

It is known that chaperone complexes with high interaction strength are characteristic of tumor cells. Since the 2000s, researchers have been actively identifying and describing HSP70/90/110 complexes with AHA, JAK, BAG, HOP, BiP, and others in solid and hematological tumor cells [8, 21, 24, 25]. Unlike more dynamic complexes of normal cells, chaperomes isolated from tumors remain stable during in vitro studies. The inclusion of chaperome components in such stable complexes does not depend on the level of tissue expression, origin, or genetic mutations [6, 32]. Moreover, it was found that, despite their activity, such HSP complexes represent only a small part of the chaperome of a tumor cell, and not all isolated cultures are able to express them, which may serve as a basis for di-

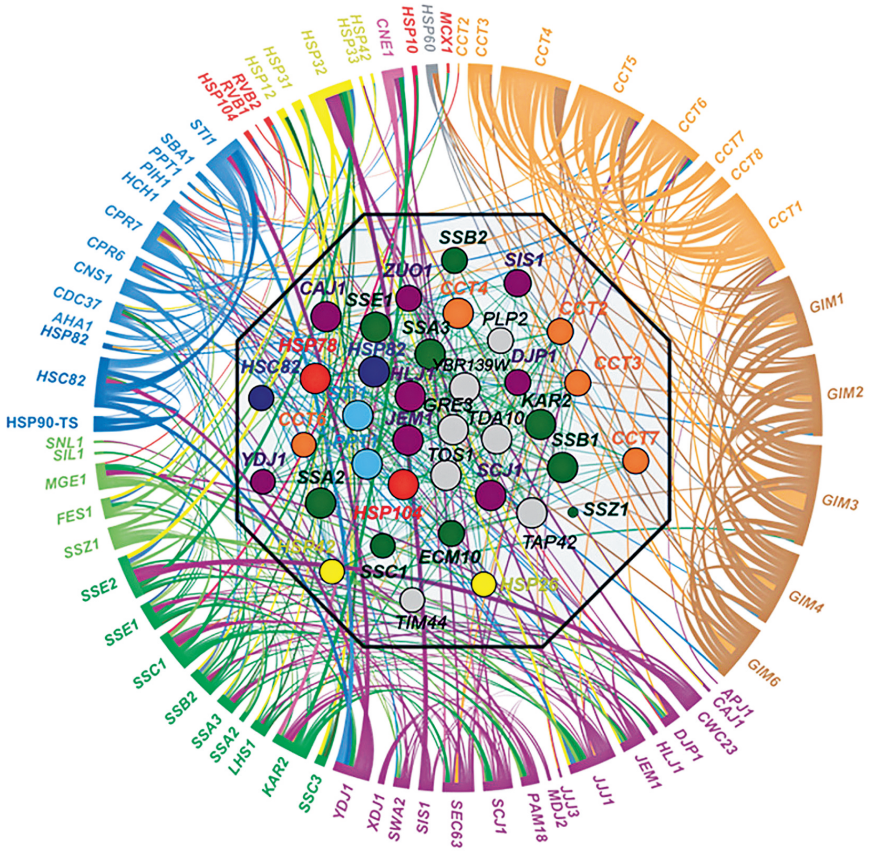

Fig. 2. An example of chaperome obtained via immunoassays and bioinformatic analysis [33]
Рис. 2. Пример шаперома, полученного иммуноферментным и биоинформатическим анализом [33]

viding cancer cell cultures into two types. Cancer cells require the formation of stable protein networks and maintenance of proteostasis. The chaperome is the main platform supporting the synthesis, organization, and protection of polypeptide pathways, also mediating signaling, transport, and intercellular contact [26, 35, 39]. This set of functions places the chaperone in the central position of the protein network, surrounding it with lower-order protein complexes and auxiliary molecules. This location is important in the diagnosis and treatment of cancer and opens up a chaperomic approach in personalized medicine: labeling or inhibiting the HSP protein knot with a high degree of connectivity is more likely to lead to the detection or apoptosis of cancer cells than targeting individual chaperones or dynamic complexes. It is this integrated approach that guides modern chaperome research both in the framework of cell biology and cancer theranostics.

ADDITIONAL INFORMATION

Author contribution. Thereby, all authors made a substantial contribution to the conception of the study, acquisition, analysis, interpretation of data for the work, drafting and revising the article, final approval of the version to be published and agree to be accountable for all aspects of the study.

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MYCOPLASMAS. BIOLOGICAL PROPERTIES (LECTURE)

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Abstract. Mycoplasmas are a unique group of prokaryotes, a characteristic feature of which is the absence of a cell wall. The features of mycoplasmas also include a minimal set of organelles, the presence of sterols in the cytoplasmic membrane, which microorganisms themselves cannot synthesize, the smallest known self-replicating genome structure, as well as membrane parasitism. The growing interest in these microorganisms is due to a number of factors: the variety of biological properties, the undoubted relevance of the pathology caused by them and many unsolved problems in the world health system. Mycoplasmas have received the greatest importance as pathogens of urogenital and respiratory infections, however, a wide range of virulence factors of these microorganisms, features of their interaction with the cellular and humoral immunity of the host causes damage to other organs associated with autoimmune mechanisms and hypersensitivity of the macroorganism. There is information about possible involvement of mycoplasmas in the process of carcinogenesis through the release of the DnaK protein, which impairs the ability of a mycoplasma-infected cell to repair DNA damage by reducing the activity of important cellular proteins such as p53. The ecological plasticity of mycoplasmas, a wide range of hosts and their ubiquity, which actualizes the problem of mycoplasma infections for almost any geographical region.

Key words: *Mycoplasmas; Mycoplasma pneumoniae; Mycoplasma genitalium; Mycoplasma hominis; Ureaplasma urealyticum;* biological properties; virulence factors.

МИКОПЛАЗМЫ. БИОЛОГИЧЕСКИЕ СВОЙСТВА (ЛЕКЦИЯ)

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Резюме. Микоплазмы представляют собой уникальную группу прокариот, отличительным признаком которых является отсутствие клеточной стенки. К особенностям микоплазм относятся также минимальный набор органоидов, наличие стеролов в составе цитоплазматической мембраны, которые сами микроорганизмы синтезировать не могут, наименьший из известных самореплицирующихся структур геном, а также мембранный паразитизм. Возрастающий интерес к этим микроорганизмам обусловлен целым рядом факторов: разнообразием биологических свойств, несомненной актуальностью вызываемой ими патологии и многими нерешенными задачами в системе мирового здравоохранения. Наибольшую значимость микоплазмы получили как возбудители урогенитальных и респираторных инфекций, однако широкий спектр факторов вирулентности, присущих этим микроорганизмам, и особенности их взаимодействия с клеточным и гуморальным иммунитетом хозяина обусловливают поражение других органов и систем, связанное с аутоиммунными механизмами и аллергической перестройкой организма. Последние данные говорят о возможном участии микоплазм в процессе канцерогенеза за счет высвобождения белка DnaK, который нарушает способность инфицированной микоплазмой клетки восстанавливать повреждения ДНК за счет уменьшения активности важных клеточных белков, таких как p53. Экологическая пластичность микоплазм обусловливает широкий круг хозяев и их повсеместное распространение, что делает проблему микоплазменных инфекций крайне актуальной практически для любого географического региона.

Ключевые слова: микоплазмы; *Mycoplasma pneumoniae*; *Mycoplasma genitalium*; *Mycoplasma hominis*; *Ureaplasma urealyticum*; биологические свойства, факторы вирулентности.

INTRODUCTION

Mycoplasmas represent an evolutionarily distinct group of microorganisms characterized by the absence of a rigid cell wall. The increasing interest in this group of prokaryotes is driven by the uniqueness of their biological properties, the undeniable clinical significance of the diseases caused by them, and a number of unresolved issues in global healthcare. Mycoplasmas are the smallest prokaryotes capable of independent reproduction. They belong to the class *Mollicutes* ("soft skin") and evolved by a regressive reduction of the genome of ancestral Gram-positive bacteria [5]. Mycoplasma genome is the smallest among all known self-replicating structures (450–500 mD), which makes these microorganisms an extremely attractive and convenient model for genetic and molecular biological research (including transcriptomic and proteomic studies). Another interesting feature of the mycoplasma genome is a deviation from the universal genetic code: in these microorganisms, the TGA triplet (normally a stop codon) codes for tryptophan. Mycoplasmas are widely distributed in nature. Their large host range makes them ubiquitous microorganisms capable of infecting various types of plants and animals (insects, amphibians, fish, birds, and mammals), including humans. Many species exist as saprophytes in soil and water. In human pathology, mycoplasmas are most significant as pathogens of infections of the urogenital tract and respiratory system, but the spectrum of diseases associated with them is much broader. Today, these microorganisms are considered cofactors in numerous pathological conditions and syndromes, including rheumatoid arthritis, Crohn's disease, and others. Mycoplasmas from various sources can spontaneously contaminate cell cultures used in virology, significantly complicating the production of vaccines and diagnostic reagents [10]. In the etiology of pneumonia, especially among school-aged children, *Mycoplasma (Mycoplasmoides) pneumoniae* often takes the leading position, accounting for 18–44% of cases during epidemics in recent years. There is no official registration of respiratory mycoplasmosis in the Russian Federation, but according to the World Health Organization, in various countries, *M. pneumoniae* accounts for about 21% of respiratory diseases in children aged 5 to 14. Moreover, it has been proven that in addition to respiratory tract infections, mycoplasmas can serve as triggers for autoimmune rheumatic diseases and allergic disorders (bronchial asthma, Stevens–Johnson syndrome), and, in combined infections with acute respiratory viral infections and herpes infections, can also be implicated in hemorrhagic vasculitis. In adults, along with classic sexually transmitted infections (STIs), there is a notably higher prevalence of urethritis and cervicitis in certain social groups caused by "new-generation" pathogenic microorga-

nisms, including *Mycoplasma (Mycoplasmoides) genitalium*. Most mycoplasmas, including *Mycoplasma (Metamycoplasma) hominis* and *Ureaplasma urealyticum*, are not absolute pathogens. Transmitted sexually, they can cause infectious and inflammatory processes in the urogenital organs under certain conditions, often in association with other pathogenic or conditionally-pathogenic microorganisms. Consequently, many authors describe mycoplasmas as "microorganisms in the service of disease" and classify them as resident microorganisms associated with STIs [8]. Currently, the global situation concerning mycoplasma infections affecting both the reproductive system and the respiratory system is complicated by the emergence and spread of *M. genitalium* and *M. pneumoniae* strains resistant to macrolides [11, 15] and fluoroquinolones [1, 27], which are widely used to treat conditions associated with mycoplasmas. In the Russian Federation, mycoplasmas and the infections they cause present certain challenges for clinical diagnostic laboratories and practicing physicians— specifically, in interpreting laboratory results and clinical manifestations, as well as in selecting adequate treatment strategies given growing antibiotic resistance of these pathogens.

HISTORY

The term "mycoplasma" (from the Greek μύκης, *mykes* — fungus, and πλάσμα, *plasma* — formed) was first used in 1889 to describe a modified state of plant cell cytoplasm resulting from the penetration of fungus-like microorganisms. For a long time, mycoplasmas could not be detected by microscopic or cultural methods. In 1898, researchers at the Pasteur Institute isolated a pathogenic microorganism [19], now known as *Mycoplasma mycoides* (part of the pleuropneumonia-like organism group) [17, 20]. This pathogen causes pleuropneumonia in cattle, characterized by severe lesions of the pleura and pulmonary parenchyma, with serous inflammation of the interlobular connective tissue and accumulation of exudate in the pleural cavity. In calves, *M. mycoides* can cause arthritis; in pigs, serous-catarrhal inflammation of the lungs and bronchi; in goats and sheep, lesions of the joints, eyes, and mammary glands. Later, it became clear that the pathogen passes through bacterial filters and does not grow on simple media (it can only be cultivated on complex serum-containing media). Today, *Mycoplasma mycoides* is included on the list of highly dangerous animal pathogens and is strictly quarantined. Next significant stage in the study of mycoplasmas and mycoplasmosis occurred in 1910, when the morphology of *Mycoplasma mycoides* was clarified [8, 22]. Nineteen years later, in 1929, the term "mycoplasmas" was proposed to indicate a group of certain filamentous microorganisms [8], which were believed (at the time) to have both cellular and acellular stages in their life cycle. This could have explained how they, while being visible under a microscope, were at the same time able to pass through bacterial filters. In 1937, *M. hominis* was isolated for the first time from an abscess of the Bartholin's gland [8]. A year later, in 1938, first cases of atypical pneumonia were described in Philadelphia; this pneumonia did not respond to sulfonamide therapy [6]. The findings were published in the *Journal of the American Medical Association* (JAMA). The disease was observed in adults and began as a mild infection, progressing to severe diffuse pneumonia with signs of encephalitis. The main clinical symptoms were shortness of breath, cyanosis, hoarseness, a non-productive cough, drowsiness, and profuse sweating. Fever lasted on average for about three weeks, and in most cases the disease resolved with recovery. First steps in studying the immunology of mycoplasma infections were taken in 1943 when a rise in antibody titers against mycoplasma antigens was noted in the cold agglutination test among patients with symptoms of atypical pneumonia [21]. This became the first available diagnostic test. In 1944, three strains of the atypical pneumonia agent ("walking pneumonia") were obtained by infecting chicken embryos with sputum from patients. The pathogen passed through bacterial filters and was designated as the "Eaton agent," considered a virus of atypical pneumonia [18]. The mycoplasma nature of this disease in humans was established after the etiological agent, called *M. pneumoniae,* was isolated on a specialized growth medium (Hayflick medium) [13], and its pathogenicity was confirmed by infecting volunteer with a pure culture of the microorganism [12]. Eventually, the ability to culture the pathogen on serum agar confirmed its bacterial nature and simplified the development of diagnostic preparations for serological tests [13]. In 1963, the atypical pneumonia agent was formally classified as a mycoplasma [8]. In 1954, *U. urealyticum* was isolated from the urethra of a patient with nongonococcal urethritis [8, 23], but its etiological role in urogenital pathology was established in volunteer studies only much later [25]. In the 1990s, genomes of several mycoplasmas were deciphered. In 1995, shortly after *Haemophilus influenzae* became the first microorganism with a fully sequenced genome, the genome of *M. genitalium* (the smallest bacterial genome) was also sequenced [9]. In 1996, the genome of *M. pneumoniae* was read, and in 2001, the nucleotide sequence of the *U. urealyticum* genome was determined [6]. Studying these genomes formed the basis of modern molecular-biological diagnostic methods for mycoplasmosis. Key historical milestones in the study of mycoplasma infections are presented in Table 1.

TAXONOMY AND BIOLOGICAL PROPERTIES OF MYCOPLASMAS

A phylogenetic tree constructed based on 16S rRNA analysis allows us to explore certain aspects of the evolution of Mollicutes. It is believed that mycoplasmas diverged from the streptococcal branch of Gram-positive bacteria about

Table 1

History of the study of mycoplasma infections

Таблица 1

История изучения микоплазменных инфекций

Год / Year	Событие / Event	Авторы / Authors
1898	Первое описание возбудителя атипичной плевропневмонии крупного рогатого скота (впоследствии *Mycoplasma mycoides*) / The first description of the causative agent of atypical pleuropneumonia in cattle (later *Mycoplasma mycoides*)	E. Nocard, E. Roux
1910	Уточнение морфологии описанных микроорганизмов / Clarification of the morphology of the described microorganisms	J. Bordet
1929	Название «микоплазмы» / Name "*mycoplasma*"	J. Nowac
1937	Выделение *Mycoplasma hominis* из абсцесса большой вестибулярной железы / Isolation of *Mycoplasma hominis* from an abscess of the great vestibular gland	Dienes Edsall
1938	Первые случаи атипичной пневмонии у человека / First cases of atypical pneumonia in humans	H. Reimann
1943	Выявление антител к микоплазмам в реакции агглютинации / Detection of antibodies to mycoplasmas in agglutination test	J. Peterson
1944	Агент Итона (возбудитель атипичной пневмонии) / Eaton's agent (causative agent of atypical pneumonia)	M. Eaton
1954	Выделение Т-микоплазмы (*Ureaplasma urealyticum*) из уретры больного негонорейным уретритом / Isolation of T-mycoplasmas (*Ureaplasma urealyticum*) from the urethra of a patient with nongonorrheal urethritis	M. Shepard
1963	Название *Mycoplasma pneumoniae* / Name *Mycoplasma pneumoniae*	R.M. Chanock
1995	Секвенирование генома *Mycoplasma genitalium* / Genome sequencing *Mycoplasma genitalium*	
1996	Секвенирование генома *Mycoplasma pneumoniae* / Genome sequencing *Mycoplasma pneumoniae*	
2001	Секвенирование генома *Ureaplasma urealyticum* / Genome sequencing *Ureaplasma urealyticum*	

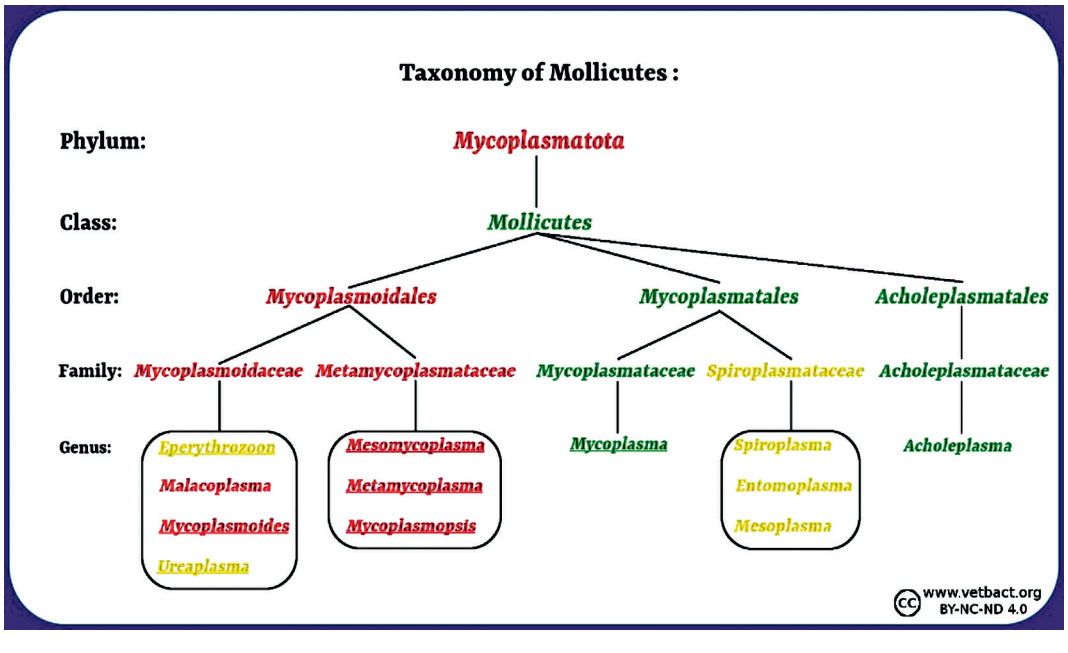

Fig. 1. Modern classification of mycoplasmas (the source: http://www.vetbact.org/displayextinfo/136)
Рис. 1. Современная классификация микоплазм (источник: http://www.vetbact.org/displayextinfo/136)

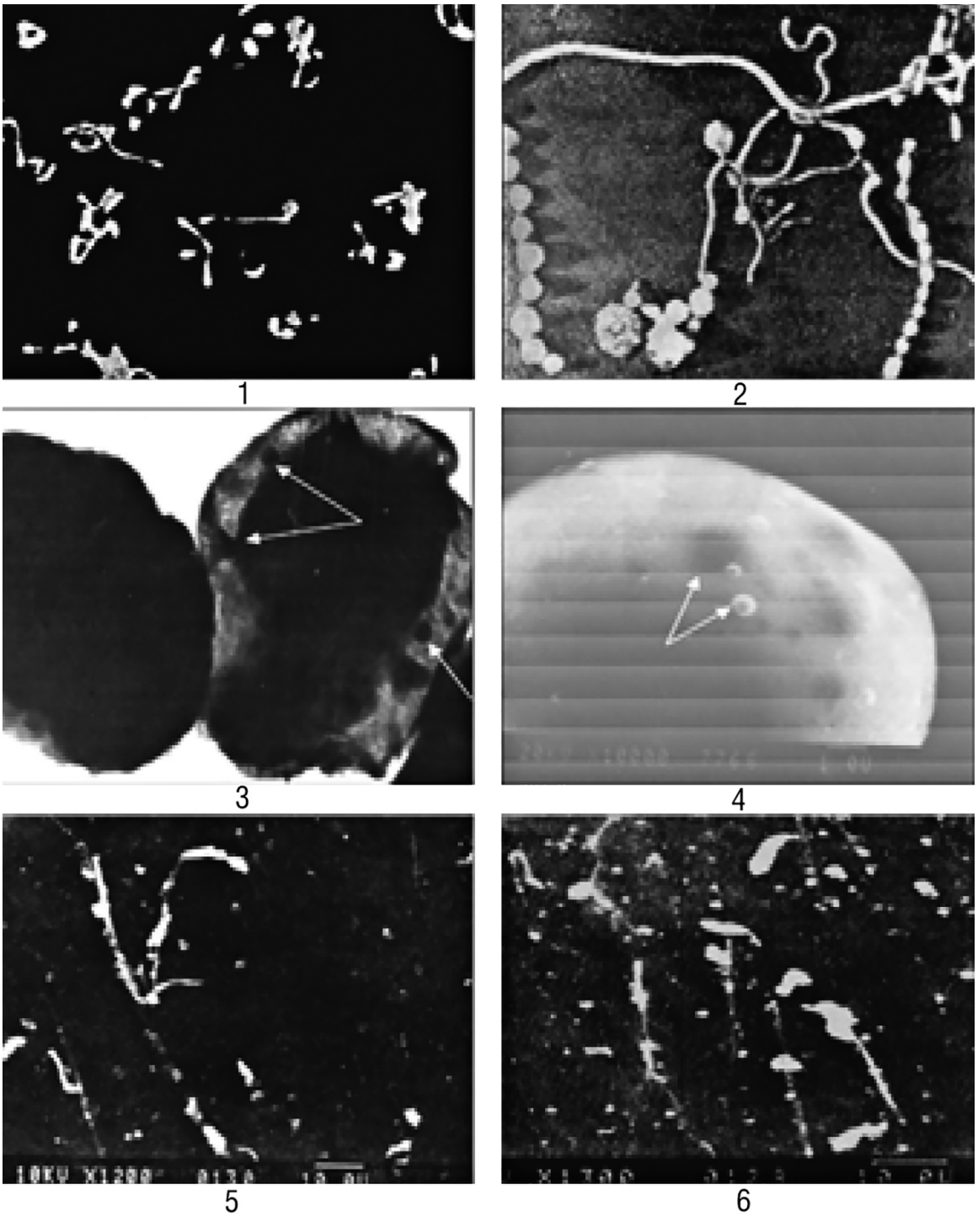

Fig 2. **Morphology of mycoplasmas (Katola V.M., 2018). Scanning electron microscopy: 1 — rat bronchopneumonia *mycoplasma* growing in a nutrient solution (micrograph by E. Kleinberger-Nobel, 1955); 2 — *M. mycoides* (according to Brock, 1970, ×20 000); 3–4 — mycoplasmas inside and on the surface of *Penicillium canescens* spores (drawing V.M. Katola, ×10 000); 5–6 — elementary bodies of L-form bacteria and filamentous forms in the blood plasma of a patient with progressive fibrous-cavernous pulmonary tuberculosis (×1200 and 1300, respectively)**

Рис. 2. **Морфология микоплазм (Катола В.М., 2018). Сканирующая электронная микроскопия: 1 — растущие в питательном растворе микоплазмы бронхопневмонии крыс (микрофотография Е. Клейнбергер-Нобель, 1955); 2 — *M. mycoides* (по Броку, 1970, ×20 000); 3–4 — микоплазмы внутри и на поверхности спор *Penicillium canescens* (рисунок В.М. Католы, ×10 000); 5–6 — элементарные тельца L-форм бактерий и нитевидные формы в плазме крови больного прогрессирующим фиброзно-кавернозным туберкулезом легких (×1200 и 1300 соответственно)**

65 million years ago through divergent evolution associated with a parasitic lifestyle. Mycoplasmas are classified under the phylum *Mycoplasmatota* (Fig. 1). This phylum is represented by a single class, *Mollicutes*, which includes three orders: *Mycoplasmatales*, *Mycoplasmoidales*, and *Acholeplasmatales*. Within the order *Mycoplasmoidales* is the family *Mycoplasmoidaceae*, which contains the genera *Mycoplasmoides* (species *M. pneumoniae*, *M. genitalium*) and *Ureaplasma* (species *U. urealyticum*, *U. parvum*). The order *Mycoplasmoidales* also includes the family *Metamycoplasmataceae*, which include the genus *Metamycoplasma* (species *M. hominis*). These microorganisms are of primary medical importance, although more than 255 species of mycoplasmas and 11 species of ureaplasmas have been described to date. Accordingly, under the current classification, mycoplasmas and ureaplasmas belong to different orders and different families.

Morphological Properties

A unique morphological feature that distinguishes mycoplasmas from other prokaryotes is the absence of a rigid cell wall [7]. This determines a number of their biological properties, particularly their polymorphism (Fig. 2). Mycoplasmas exhibit large and small spherical forms, elliptical or discoid shapes, flask-like structures, rod-shaped or filamentous branching forms of various lengths, and other unusual morphologies [4]. Their polymorphism is related to the lack of peptidoglycan or any substitutes that stabilize cell shape. These bacteria are surrounded only by a three-layer cytoplasmic membrane, which maintains the cell's osmotic integrity but does not provide a fixed form. Unlike other prokaryotes, mycoplasmas have a high sterol content (e.g., cholesterol) in their cytoplasmic membrane, which they are unable to synthesize on their own. These sterols provide stability, rigidity, and strength to the membrane. Absence of peptidoglycan also determines natural resistance to beta-lactam antibiotics. Mycoplasma pathogens are the smallest bacteria, with sizes ranging from 0.1 to 0.6 micrometers, enabling them to pass through bacterial filters with a pore diameter of 0.22 micrometers. Mycoplasmas have a minimal set of organelles: only a cytoplasmic membrane, a nucleoid, and ribosomes. They do not form spores, do not have flagella, and some species can form a microcapsule. When Gram-stained, they appear Gram-negative.

Despite absence of flagella, certain mycoplasmas are capable of movement. For a long time, it was assumed that bacteria, unlike eukaryotic cells, do not have a cytoskeleton. However, later research revealed that cytoskeleton-like structures form during division and growth in almost all bacteria, including mycoplasmas. These cytoskeletal structures can enable motility. For instance, spiroplasmas, which have a spiral shape, can bend, crawl, and swim by twisting like a corkscrew, but unlike spirochetes, they do not possess endoflagella. Instead, they rely on special protein filaments twisted into a spiral, whose secondary structure is provided by an actin-like protein. Among mycoplasmas, there are both motile and non-motile variants. Motile forms move by sliding along solid surfaces. *Mycoplasma (Mesomycoplasma) mobile* is the fastest species, moving across glass surfaces at speeds of 2.0–4.5 μm per second. The cytoskeleton of this microorganism resembles a jellyfish in appearance.

Most mycoplasma species present a low G+C ratio in their DNA (about 30%), with the exception of *M. pneumoniae*, which has a G+C content of 38.6–40%. *U. urealyticum* has the lowest G+C ratio among all known bacterial genomes (25.5%). The theoretical minimum G + C content necessary to encode proteins with the normal set of amino acids is about 26%; for this reason, mycoplasmas stand at the "edge of life".

Cultural and Biochemical Properties

Mycoplasmas are generally facultative anaerobes, except for *M. pneumoniae*, which is a strict aerobe. The minimal genetic information in mycoplasmas translates into a minimal number of metabolic pathways, explaining their dependence on host cells. All mycoplasmas studied to date are characterized by shortened respiratory chains with flavin terminals, excluding oxidative phosphorylation as a mechanism for ATP generation. It is thought that non-fermenting mycoplasmas use arginine breakdown via the arginine dihydrolase pathway as their main source of ATP. Ureaplasmas have a unique requirement for urea that is not observed in other living organisms. Because they are neither glycolytic nor have an arginine dihydrolase pathway, it was hypothesized (and later experimentally confirmed) that ATP is generated via an electrochemical gradient created by ammonia released during the intracellular hydrolysis of urea by urease.

A variety of reproductive mechanisms have been described in mycoplasmas (fragmentation, binary fission, budding). A portion of the newly formed cells turn out to be nonviable. As noted, mycoplasmas are the smallest known cellular organisms—some even smaller than the theoretical threshold for independent cell reproduction on nutrient media (0.15–0.20 μm for spherical cells and 13 μm in length and 20 nm in diameter for filamentous forms). The limited biosynthetic capabilities of mycoplasmas determine their extreme nutritional and cultural demands. Enriched media containing precursors of nucleic acids, proteins, and lipids are essential for their growth. Mycoplasmas, in particular, are highly dependent on sterols (cholesterol and its derivatives) and fatty acids, with cholesterol dominating among membrane lipids and providing stability to the cytoplasmic membrane. In the infected host, mycoplasmas obtain

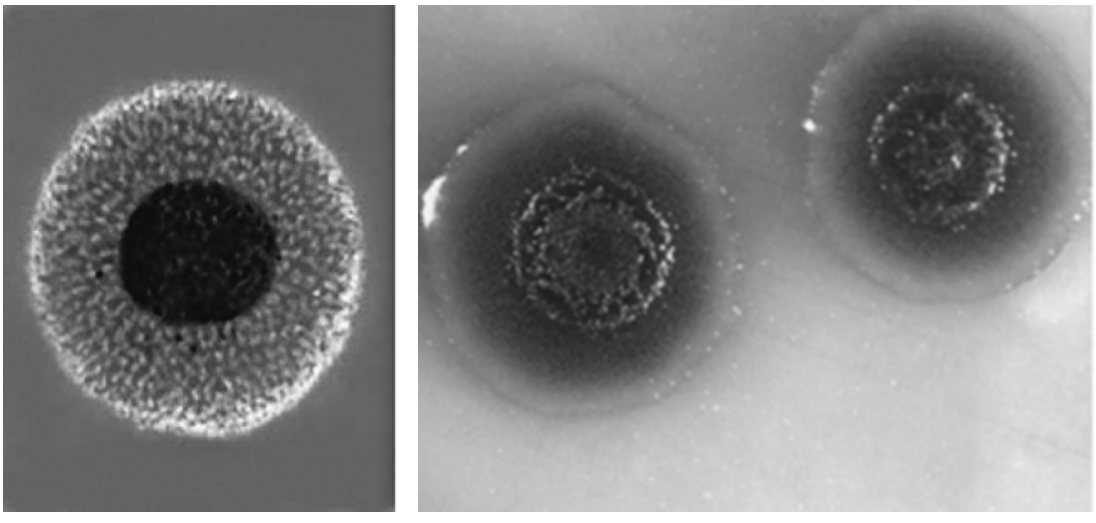

Fig. 3. Colonies of mycoplasmas, similar to fried eggs. Microphotography, magnification ×100
Рис. 3. Колонии микоплазм, похожие на яичницу-глазунью. Микрофотография, увеличение ×100

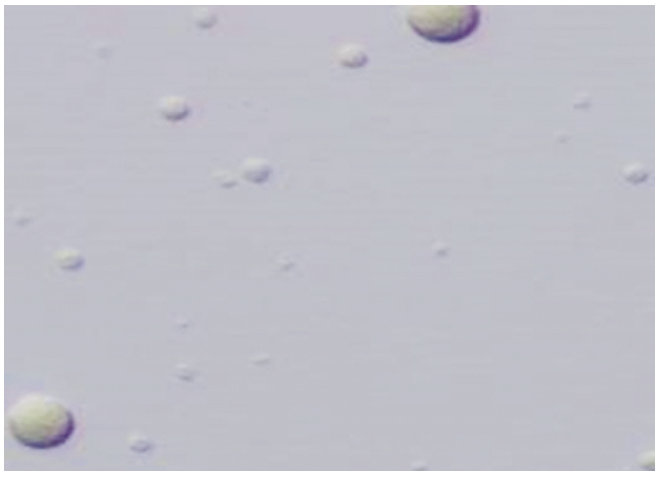

Fig. 4. Spherical colonies of *M. genitalium* after 10 days of incubation. Microphotography, magnification ×100 (Ken B. Waites et al., 2023
Рис. 4. Сферические колонии *M. genitalium* после 10 дней инкубации. Микрофотография, увеличение ×100 (Ken B. Waites et al., 2023)

sterols from host cells, which justifies their classification as "membrane parasites". It has been demonstrated by confocal electron microscopy that they can fuse with and invade into host cell membranes. For cultivation, media enriched with horse serum, yeast extract, arginine, urea, glucose, vitamins, and amino acids are used. Mycoplasmas grow slowly: *U. urealyticum* colonies appear within 24–48 hours, while other species may take 3–5 days. The optimal temperature is 37°C. To suppress contaminating flora, penicillin and its analogs are used for mycoplasmas or lincomycin for ureaplasmas. Most mycoplasmas grow well in an atmosphere of 95% nitrogen and 5% carbon dioxide.

Because of their small size and absence of a rigid cell wall, most mycoplasmas can penetrate the spaces between agar fibers and multiply below the agar surface. After 18 hours, a tiny spherical colony forms beneath the surface; by 24–48 hours of incubation, it reaches the surface water film, creating two zones of growth—a turbid granular center ingrown in the agar and a flat, translucent, lacy periphery—giving colonies their characteristic "fried egg" appearance (Fig. 3). Colonies are small, measuring 0.1–0.3 mm for most mycoplasmas (Fig. 4), and ureaplasma colonies are even smaller.

In semi-solid agar, colonies appear as small pinfeathers. In broth, mycoplasmas exhibit opalescence; in contrast, broth cultures of ureaplasmas remain clear, and their growth is detected by a change in the color of the indicator.

Mycoplasmas have low biochemical activity, which varies by species and strain. Currently, two groups of mycoplasmas are distinguished:

1. Those that ferment glucose, maltose, mannose, fructose, starch, and glycogen to form acid.
2. Those that reduce tetrazolium compounds, oxidize glutamate and lactate, but do not ferment carbohydrates.

Members of the genus *Ureaplasma* spp. exhibit high urease activity and degrade urea, are inert to sugars, do not reduce diazo dyes, and are catalase-negative. An important feature of mycoplasma metabolism is the ability to produce saturated and unsaturated fatty acids. For the differentiation of isolated mycoplasma strains, it is extremely important to determine a range of biochemical characteristics such as phosphatase, proteolytic, and phospholipase activity. In addition, tests for tetrazolium reduction and reactions with erythrocytes are used, along with other biochemical assays (Table 2).

Table 2

Biochemical properties of mycoplasmas

Таблица 2

Биохимические свойства микоплазм

Виды микоплазм / Mycoplasmas spp.	Метаболизм / Metabolism			Пленки на поверхности среды / Films on the surface of the medium	Фосфатазная активность / Phosphatase activity	Гидролиз казеина / Casein hydrolysis	Редукция тетразолиума / Tetrazolium reduction	Взаимодействие с эритроцитами / Interaction with red blood cells	
	глюкозы / glucose	аргинина / arginine	мочевины / urea					Гемадсорбция / Hemad sorption	Гемолиз и гемагглютинация / Hemolysis and hemagglutination
M. pneumoniae	+	–	–	–	–	–	+	+	+
M. hominis	–	+	–	–	–	–	–	–	–
M. genitalium	+	–	–	–	–	–	Слабая в аэробных условиях, в анаэробных — отсутствует / Weak under aerobic conditions, in anaerobic — absent	+	–
M. fermentans	+	+	–	+	+	–	В аэробных условиях –, в анаэробных + / Under aerobic conditions –, under anaerobic +	–	β-гемолиз эритроцитов барана – / β-hemolysis of sheep erythrocytes –
M. penetrans	+	+	–	–	+	–	В аэробных условиях +, в анаэробных – Under aerobic conditions +, under anaerobic conditions –	+	Слабые / Weak
Ureaplasma spp.	–	–	+	–	+	+	–	+	Эритроциты кролика +, морской свинки + / Erythrocytes of a rabbit +, guinea pig +

Antigenic Properties

Mycoplasmas have a complex, polymorphic antigenic structure that differs by species and is defined by a high frequency of spontaneous and induced mutations. Because they lack a cell wall, the principal antigens of these microorganisms are found in the cytoplasmic membrane and certain surface structures. Mycoplasmal membrane antigens are numerous and diverse. Chemically, they include proteins, polysaccharides, and lipids. The most immunogenic are surface antigens containing carbohydrates as part of complex glycolipid, lipoglycan, or glycoprotein complexes. Antigenic structure may change after multiple passages in cell-free media. For example, *M. hominis* contains more than nine integral membrane proteins, of which only two are consistently present in all strains. More than 16 serovariants of ureaplasmas have been identified, differing in the antigenic structure of their surface polypeptides. Notably, some mycoplasma species have a polysaccharide capsule, underlining the antigenic diversity of these microorganisms and contributing to resistance against phagocytosis. Certain membrane antigens of mycoplasmas have been studied and characterized, including the P1 antigen of *M. pneumoniae* (molecular weight 168 kDa) and Pa antigen of *M. genitalium* (140 kDa). These antigens in their respective species are major immunogens. Cytoplasmic antigens are less diverse and less immunogenic compared to membrane antigens, exhibiting similarities across different mycoplasma species; for that reason, they are not used for immunoserum production or identification. Some mycoplasmal

antigens resemble human cell and tissue components and induce various immunomodulatory effects (superantigenicity), which undoubtedly play a role in mycoplasma virulence and the pathogenesis of the infections they cause.

Virulence Factors

The pathogenicity of mycoplasmas is currently a topic of active debates in numerous publications on their virulence and its contributing factors. The frequent detection of *M. hominis* and *U. urealyticum* in asymptomatic individuals complicates the question of their etiological and pathogenetic role. *M. pneumoniae* and *M. genitalium* are unconditionally accepted as pathogenic, whereas *M. hominis* and *U. urealyticum* are considered conditionally-pathogenic and can cause infection under certain conditions. Most of the remaining mycoplasma species likely exist as harmless commensals of mucous membranes. At the same time, there is evidence that mycoplasmas release the DnaK protein, one of the chaperone family proteins [28]. This protein impairs an infected cell's ability to repair DNA damage by reducing the activity of key cellular proteins (e.g., p53) that are involved in DNA repair and tumor suppression, as a result increasing the risk of cancer. Additionally, DnaK may enter neighboring uninfected cells. By suppressing p53, DnaK can also reduce the efficacy of anticancer drugs [28], highlighting the complex and ambiguous nature of host–mycoplasma interactions and the importance of further research.

Mycoplasmas are membrane parasites. Their key virulence factor is the ability to attach to host cells. Some species possess specialized organelles in which adhesin proteins, necessary for cell binding, are structurally and functionally co-localized. In other species, specialized organelles are absent, and the function of adhesins is performed by any areas of the cell surface containing the corresponding proteins. For example, in *M. pneumoniae* and *M. genitalium*, P1 and P140 proteins, respectively, perform this function. Within 24 hours of infection, *M. pneumoniae* begins to adhere to the respiratory epithelium. This mechanism protects the microorganism from mucociliary clearance and is considered the onset of disease. Mycoplasma has an "attachment organelle" that not only binds tightly to the host cell but also enables gliding motility. By penetrating between the cilia, it induces desquamation of epithelial cells. Recently, the unique gliding mechanism of mycoplasmas and the structure of the "attachment organelle" have been described. This organelle is a membrane protrusion at the anterior pole of the cell composed of 15 proteins, with the P1 adhesin (168 kDa) on the surface.

The gliding speed of *Mycoplasma* averages 0.2–0.5 μm/s but can reach 1.5–2 μm/s, meaning the microorganism traverses the length of a cell within one second. Certain mycoplasma adhesins are heterogeneous in structure and function. For instance, based on some properties of the P1 protein, mycoplasmas are subdivided into eight groups, which may underlie variations in strain pathogenicity. In addition to these proteins, other molecules such as P32, HMW1, HMW2, and HMW3 (in *M. genitalium*), lipoproteins P120, P50, and P60 (in *M. hominis*), and the MVA protein (in *U. urealyticum*) have been described. Mycoplasma adhesins are rich in proline, which increases cell binding, and function as immunogens. Mycoplasmas interact with multiple receptor types on the host cell: sialylated oligosaccharides, with which they have high affinity (abundant on epithelial cells), non-sialylated glycoproteins, and sulfated glycolipids. A very important and interesting feature of mycoplasmas is their ability to cause hemolysis when adsorbed onto erythrocytes, likely via hydrogen peroxide release (with the possible exception of ureaplasmas). *M. pneumoniae* exhibits the highest hemolytic activity. In most other pathogenic bacteria, hemolysins are protein or lipid in nature. This underscores the uniqueness of mycoplasmas and their significant adaptive capacity, despite their limited genome.

Penetration and adhesion are undoubtedly fundamental steps in the infectious process, as they control the further development of disease. However, just high adhesive capability alone would not allow mycoplasmas to overcome the cellular–tissue barrier and immune defenses. Some mycoplasma species can produce invasive enzymes that destroy cells. For instance, mycoplasmas produce neuraminidase, which affects cellular receptors and intercellular contacts. Various proteases induce cell degranulation and degrade essential amino acids (e.g., arginine), potentially leading to apoptosis. Of particular note are IgA proteases, which degrade IgA and deprive it of its protective function. Among virulence enzymes, phospholipase A and aminopeptidases are especially significant for their ability to hydrolyze phospholipids of cell membranes, including those of the placenta and fetus (*M. hominis* and *U. urealyticum*). Other enzymes include RNases [3], DNases, and thymidine kinases that disrupt nucleic acid metabolism in host cells. Nucleic acid destruction leads to genome instability. DNases of *U. urealyticum* degrade sperm DNA; the P40 endonuclease of *Mycoplasma (Malacoplasma) penetrans* induces apoptosis in human peripheral blood lymphocytes and monocytes. It has been suggested that the pathogenesis of mycoplasmoses is associated with impaired transcription in host cells due to mycoplasmal RNA polymerases. Besides enzymes, mycoplasmas can produce metabolites with cytotoxic effects, such as ammonia and acidic byproducts, which raise pH and destroy infected cells. As mentioned earlier, hydrogen peroxide and superoxide anion generation leads to red blood cell hemolysis. There is an opinion that some

mycoplasmas can invade host cells, though the mechanisms are not fully understood. It is assumed that mycoplasmas may fuse with the cell membrane and penetrate the perinuclear region. Such mycoplasmas are called "fusogenic" — for example, *Mycoplasma (Mycoplasmopsis) fermentans*, which can reorganize the host cell cytoskeleton. It is now known that mycoplasmas possess protein substances (referred to in the literature as "mycoplasma endotoxins") that damage the ciliated epithelium of the respiratory tract and inactivate neutrophils. Such substances have been described in *M. pneumoniae* and *M. fermentans*. Over the past decade, research into the pathogenicity of *M. pneumoniae* has led to the discovery of a unique mycoplasmal CARDS toxin (Community Acquired Respiratory Distress Syndrome toxin) that causes vacuolization of bronchial epithelial cells and reduces ciliary motility. CARDS toxin directly damages the respiratory epithelial cells, causing extensive peribronchial and perivascular inflammation. A direct correlation has been found between the amount of CARDS toxin secreted by *M. pneumoniae* and the severity of lung tissue damage [2]. Interestingly, CARDS toxin shares similarities with *Bordetella pertussis* exotoxin [16, 24]. The cytotoxic effects of CARDS toxin manifest in catarrhal symptoms observed in acute respiratory viral infections. There have been reports of fulminant mycoplasma infection with severe respiratory failure and acute respiratory distress syndrome (ARDS) in very young children [2] and elderly patients, presumably associated with the action of CARDS toxin [2, 26]. Experiments have shown that recombinant CARDS

Table 3

***Mycoplasma* virulence factors**

Таблица 3

Факторы вирулентности микоплазм

Факторы вирулентности / Virulence factors	Вызываемый эффект / Effect caused
Адгезины (P1 и др.) / Adhesins (P1, etc.)	Прикрепление к клеткам / Attachment to cells Мембранный паразитизм / Membrane parasitism
Нейраминидаза / Neuraminidase	Действие на рецепторы клеток и межклеточные контакты / Effect on cell receptors and intercellular contacts
Фосфолипаза А / Phospholipase A	Разрушение мембран клеток / Destruction of cell membranes
IgA-протеаза / IgA-protease	Расщепление IgA, снижение защитной функции / IgA breakdown, decreased protective function
Протеазы / Protease	Дегрануляция клеток, расщепление незаменимых аминокислот / Cell degranulation, breakdown of essential amino acids
ДНК-аза / DNAase	Дестабилизация клеточного генома, разрушение ДНК сперматозоидов, индукция апоптоза / Destabilization of the cellular genome, destruction of sperm DNA, induction of apoptosis
РНК-аза / RNAase	Нарушение процессов транскрипции в клетках / Disruption of transcription processes in cells
Токсичные метаболиты (аммиак, кислоты)/ Toxic metabolites (ammonia, acids)	Повышение pH, деструкция клеток / Increased pH, cell destruction
Гемолизины (перекись водорода, супероксидные анионы) / Hemolysins (hydrogen peroxide, superoxide anions)	Гемолиз эритроцитов / Hemolysis of red blood cells
Белковые субстанции («эндотоксины» микоплазм) / Protein substances ("endotoxins" of mycoplasmas)	Повреждение ресничек эпителия, дезактивация нейтрофилов / Damage to epithelial cilia, deactivation of neutrophils
Антигенная мимикрия / Antigenic mimicry	Персистенция в организме, аутоиммунные процессы / Persistence in the body, autoimmune processes
Суперантиген / Superantigen	Иммунные повреждения клеток и тканей цитокинами / Immune damage to cells and tissues by cytokines
Экзотоксин CARDS-токсин (community acquired respiratory distress syndrome toxin) / Exotoxin (CARDS-toxin)	Цитотоксическое действие на эпителий респираторного тракта, аллергизация / Cytotoxic effect on the epithelium of the respiratory tract, allergization

toxin can induce a potent allergic inflammation in the lungs and hyperproduction of cytokines, suggesting a possible role for *M. pneumoniae* in the pathogenesis of bronchial asthma [2, 14]. Mycoplasmas can persist for long periods inside phagocytes (leukocytes, macrophages), thanks to the presence in some strains of a microcapsule as well as antigens that cross-react with human tissue antigens ("antigenic mimicry"). Some mycoplasmas (*Mycoplasma (Metamycoplasma) arthritidis*) produce superantigens that trigger nonspecific polyclonal lymphocyte proliferation and a massive cytokine release (interleukins 6, 8, and 12, tumor necrosis factor, etc.), leading to toxic shock, joint damage, necrosis, and secondary immunodeficiency. Numerous mycoplasma virulence factors are listed in Table 3.

CONCLUSION

Mycoplasmology is reasonably recognized as a distinct branch of medical microbiology with its own research strategies and diverse investigative methods at the core of laboratory diagnosis of mycoplasma infections. Undoubtedly, urogenital and respiratory mycoplasmas have the most clearly established clinical associations, yet the complexity and ambiguity of mycoplasma–host cell interactions suggest their potential role in many other diseases, including serious systemic pathologies. The unique morphology of these bacteria, along with their wide range of virulence factors, underlies their ecological plasticity, their capacity to cause mixed infections with other bacteria and viruses, the development of antibiotic-resistant strains, and the possibility of acting as a trigger in the development of immunopathology and oncological diseases.

Thus, continued study of the biological properties of mycoplasmas, their metabolic features, and their interactions with the host organism will not only improve methods for laboratory diagnosis of mycoplasma infections but also elucidate subtle mechanisms in the pathogenesis of numerous diseases of high clinical relevance.

ADDITIONAL INFORMATION

Author contribution. Thereby, all authors made a substantial contribution to the conception of the study, acquisition, analysis, interpretation of data for the work, drafting and revising the article, final approval of the version to be published and agree to be accountable for all aspects of the study.

Competing interests. The authors declare that they have no competing interests.

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Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

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NATALIA R. KARELINA IS AN OUTSTANDING SOVIET AND RUSSIAN ANATOMIST

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Abstract. In November 2023, Natalia R. Karelina, Doctor of Medical Sciences, Professor, Head of the Department of Human Anatomy of the St. Petersburg State Pediatric Medical University of the Ministry of Health of Russia, member of the Board of the Scientific Medical Society of Anatomists, Histologists and Embryologists of Russia, turned 80. Natalia Rafailovna is a graduate of the Leningrad Pediatric Medical University. Since 1967 he has been working as a pediatrician, and since 1970 as an assistant at the Department of Human Anatomy. In 1980 successfully defends his dissertation for the degree of Candidate of Sciences, and in 1994, Doctor of Sciences. From 1995 to 2000 Natalia Rafailovna holds the position of Professor of the Department of Morphology of the Institute of Medical Education at the Novgorod University Yaroslav the Wise, from 2000 to 2003 in charge of it. The activity of Professor Karelina at that time was not limited to the department: from 1995 to 1997. She is the vice-rector for Science, and from 1997 to 2000 — Dean of the Medical and Dental Faculties. In 2003 She was elected to the position of Head of the Department of Human Anatomy of the St. Petersburg Pediatric Medical Academy. In the period 2013–2014 he holds the post of Dean of the Faculty of Additional and Vocational Education. N.R. Karelina is the supervisor of nine PhD dissertations, scientific consultant of two doctoral dissertations. N.R. Karelina is the scientific supervisor of nine PhD theses, scientific consultant of two doctoral theses, author of more than 300 scientific publications. The founder and president of the St. Petersburg Symposium on Morphology, Biochemistry, Normal and pathological Physiology of the child, whose goals are to popularize medical science. The University Administration, the Academic Council, the St. Petersburg Branch of the Scientific Medical Society of Anatomists, Histologists and Embryologists, the Editorial Board of the journal “Russian Biomedical Research”, the staff of the Department of Human Anatomy and students cordially congratulate Natalia Rafailovna, wish her good health, inexhaustible energy and creative success for the benefit of her beloved science and her native university.

Key words: Natalia Rafailovna Karelina; Professor N.R. Karelina; St. Petersburg State Pediatric Medical University; human anatomy; morphology.

КАРЕЛИНА НАТАЛЬЯ РАФАИЛОВНА — ВЫДАЮЩИЙСЯ СОВЕТСКИЙ И РОССИЙСКИЙ УЧЕНЫЙ-АНАТОМ

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Резюме. В ноябре 2023 г. исполнилось 80 лет Наталье Рафаиловне Карелиной — доктору медицинских наук, профессору, заведующей кафедрой анатомии человека ФГБОУ ВО «Санкт-Петербургский государственный педиатрический медицинский университет» Минздрава России, члену правления Научного медицинского общества анатомов, гистологов и эмбриологов России. Наталья Рафаиловна — выпускница Ленинградского педиатрического медицинского университета. С 1967 г. работает врачом-педиатром, а с 1970 г. — ассистентом кафедры анатомии человека. В 1980 г. успешно защищает диссертацию на соискание ученой степени кандидата наук, а в 1994 г. — доктора наук. С 1995 по 2000 г. Наталья Рафаиловна занимает должность профессора кафедры морфологии Института медицинского образования в составе Новгородского университета им. Ярослава Мудрого, с 2000 по 2003 гг. заведует ею. Деятельность профессора Карелиной на тот период не ограничивается кафедрой: с 1995 по 1997 г. она является проректором по науке, а с 1997 по 2000 г. — деканом лечебного и стоматологического факультетов. В 2003 г. избрана на должность заведующей кафедрой анатомии человека Санкт-Петербургской педиатрической медицинской академии. В период 2013–2014 гг. занимает пост декана факультета дополнительного и профессионального образования. Н.Р. Карелина является научным руководителем девяти кандидатских диссертаций, научным консультантом двух докторских диссертаций, автором более 300 научных публикаций. Создатель и президент Санкт-Петербургского симпозиума по морфологии, биохимии, нормальной и патологической физиологии ребенка, в цели которого заложена популяризация медицинской науки. Администрация Университета, Ученый совет, Санкт-Петербургское отделение Научного медицинского общества анатомов, гистологов и эмбриологов, редакция журнала «Российские биомедицинские исследования», сотрудники кафедры анатомии человека и студенты сердечно поздравляют Наталью Рафаиловну, желают ей крепкого здоровья, неиссякаемой энергии и творческих успехов на благо любимой науки и родного университета.

Ключевые слова: Наталья Рафаиловна Карелина; профессор Н.Р. Карелина; Санкт-Петербургский государственный педиатрический медицинский университет; анатомия человека; морфология.

Tho' much is taken, much abides; and tho'
We are not now that strength which in old days
Moved earth and heaven, that which we are, we are,-
One equal temper of heroic hearts,
Made weak by time and fate, but strong in will
To strive, to seek, to find and not to yield.
Alfred Lord Tennysson

In November 2023 Natalia Rafailovna Karelina, Doctor of Medical Sciences, Professor, Head of the Department of Human Anatomy of the St. Petersburg State Pediatric Medical University of the Ministry of Health of Russia, member of the Board of the Scientific Medical Society of Anatomists, Histologists and Embryologists of Russia, turns 80.

Natalia Rafailovna is an outstanding Soviet and Russian scientist-anatomist, beloved teacher of many generations of graduates of our university (Fig. 1).

Career of Professor N.R.Karelina began from her student days in Leningrad Pediatric Medical Institute (LPMI), which she entered after finishing secondary school in 1961 (Fig. 2).

After the very first classes at the Anatomy Department Natalia Rafailovna fell in love with the subject once and for all. And she could not help falling in love with anatomy being

taught by brilliant Grigory Ivanovich Korchanov (Fig. 3). He was a radiologist, surgeon, excellent lecturer, expert in teaching methodology, very well educated and intelligent. His high culture, amazing modesty and kindness earned him love and respect of the staff of the Department and students. Grigory Ivanovich gave classes on radiology and headed the study group on dissection where Natalia got her first skills on how to use a scalpel and forceps. She still cherishes the memory of her first teacher reminiscing him frequently.

After graduation in 1967, Natalia Rafailovna left her native Leningrad for Belarus for a career assignment, where she worked as a pediatrician for 3 years. In 1970 there was a turning point in the life of the future professor Karelina — she returned to Leningrad. And she faced the difficult choice of her future specialty — Shall she choose pharmacology (Head of the Department was professor I.V. Markova) or anatomy? Answering the call of her heart and her fate Natalia Rafailovna chose anatomy, where the young lecturer had enjoyed love and care of the same people who had supported her during her student years (Fig. 3): L.N. Korobkova, E.N. Dolgopolova, Z.V. Galtsova, V.N. Verbitskaya, and, of course, G.I. Korchanov. The Department of Anatomy of LPMI was headed by professor Georgy Filippovich Vsevolodov at that time (from 1964 to 1977). G.F. Vsevolodov received the classical anatomical education at academician V.N. Tonkov' school. As a lecturer and methodologist Vsevolodov was a unique person, his methods of presenting the subject were specified by his distinctive personality and

Fig. 2. **N.R. Karelina — a student, together with her classmates at the cleaning of the territory (first on the right)**
Рис. 2. **Н.Р. Карелина — студентка, вместе с однокурсницами на уборке территории (первая справа)**

Fig. 1. **Professor Karelina Natalia Rafailovna, 2023**
Рис. 1. **Профессор Карелина Наталья Рафаиловна, 2023 г.**

Fig. 3. **Grigory I. Korchanov (in the center) in class with students, 1963**
Рис. 3. **Григорий Иванович Корчанов (в центре) на занятиях со студентами, 1963 г.**

Fig. 4. N.R. Karelina is a young teacher with students at the Alma mater, in the center at the table
Рис. 4. Н.Р. Карелина — молодой преподаватель со студентами в Alma mater, в центре за столом

Fig. 5. Georgy F. Vsevolodov, staff of the Department of Human Anatomy and students of the FPC, 1971
Рис. 5. Георгий Филиппович Всеволодов, сотрудники кафедры анатомии человека и слушатели ФПК, 1971 г.

artistry. Bright lecturing temperament, pitch of his voice, diction, perfect knowledge of the lecture subject attracted listeners. This formed the young lecturer Karelina's future style (Fig. 4).

Soon Georgy Filippovich Vsevolodov suggested Natalia Rafailovna a theme for her thesis within the framework of the Department's research "Vascular System in Age-Related aspect" and referred her to the Faculty of Advanced Training of the Department of Anatomy of the Second Medical Institute named after N.I. Pirogov to Academician Vassiliy Vassilyevich Kupriyanov (Fig. 5, 6).

At the laboratory of microcirculation and electron microscopy under the supervision of experienced staff and academician Kupriyanov himself Karelina acquired new techniques on how to produce preparations for research (Fig. 7).

In 1977 G.F. Vsevolodov retired having worked for LPMI for more than 20 years. From this moment, Margarita

Fig. 6. Academician V.V. Kupriyanov congratulates N.R. Karelina on being awarded the degree of Candidate of Medical Sciences

Рис. 6. Академик В.В. Куприянов поздравляет Н.Р. Карелину с присуждением ученой степени кандидата медицинских наук

Fig. 7. Students of Professor V.V. Kupriyanov at the IX Congress of Anatomists, Histologists and Embryologists (from left to right: V.V. Kulikov, V.N. Levin, N.R. Karelina, V.V. Banin)

Рис. 7. Ученики профессора В.В. Куприянова на IX съезде анатомов, гистологов и эмбриологов (слева направо: В.В. Куликов, В.Н. Левин, Н.Р. Карелина, В.В. Банин)

Alexandrovna Dolgova headed the Department (Fig. 8). Owing to Margarita Alexandrovna Natalia Rafailovna continued her study within her thesis work “Intra-organ small intestine blood stream in the early post-natal ontogenesis”. The research was completed in 1979 and in 1980 N.R. Karelina successfully defended it in front of the Dissertation Committee at the Yaroslavl Medical Institute (Fig. 6).

Having won a Candidate degree Natalia Rafailovna did not rest: her scientific work gradually grew to a doctorate thesis on which Margarita Alexandrovna insisted. Thus, by M.A. Dolgova’s idea and on academician V.V.Kugriyanov’s proposal an agreement on scientific cooperation was concluded between the Department of Human Anatomy of the Leningrad Pediatric Medical Institute and the Department of Microcirculation and Electron Microscopy of the II Moscow Medical Institute. Natalia Rafailovna was attached to the department as a senior staff scientist at the Department of Microcirculation and Electron Microscopy in order to complete her doctorate research.

In 1993 N.R. Karelina was chosen for the position of a senior lecturer of the Department of Human Anatomy of the Leningrad Pediatric Medical Institute.

One year later, in 1994 Natalia Rafailovna successfully defended a doctorate thesis on “Morphogenesis, microscopic anatomy and ultrastructure of the small intestine villi (experimental-morphological study)” in the Dissertation Committee of the Russian State Medical University named after N.I. Pirogov [11, 12]. The thesis advisors were academician of RAMS, Doctor of Medical Sciences, Professor V.V. Kupriyanov and corresponding member of RAMS, Doctor of Medical Sciences, Professor A.A. Mironov.

Having got a Doctorate degree, in 1995 Natalia Rafailovna accepted the position of Professor of the Department of Morphology of the Institute of Medical Education at the Novgorod University named after Yaloslav the Wise and moved to Veliki (the Great) Novgorod.

Fig. 8. From left to right: Assistant of the department N.R. Karelina and head of the department, Professor M.A. Dolgova discussing the topics of student works of the scientific circle of the department, 1980

Рис. 8. Слева направо: ассистент кафедры Н.Р. Карелина и заведующая кафедрой, профессор М.А. Долгова обсуждают темы студенческих работ научного кружка кафедры, 1980 г.

At the institute, created almost from scratch, there was a lack of highly qualified academic and teaching staff.

Academician Mikhail Romanovich Sapin recommended Natalia Rafailovna to the University management as a highly qualified, intelligent, active and smart employee. Actively working staff is being formed at the department with

Fig. 9. **The staff of the Department of Human Anatomy together with the Vice-rector for Academic Affairs, professor V.I. Orel, 2021**
Рис. 9. **Коллектив кафедры анатомии человека совместно с проректором по учебной работе, профессором В.И. Орлом, 2021 г.**

Fig. 10. **At the meeting of the St. Petersburg branch of the NMOAGE, 2023. From left to right: Associate Professor M.V. Tvardovskaya, Professor N.R. Karelina, Associate Professor E.V. Toropkova**
Рис. 10. **На заседании Санкт-Петербургского отделения НМОАГЭ, 2023 г. Слева направо: доцент М.В. Твардовская, профессор Н.Р. Карелина, доцент Е.В. Торопкова**

intensive interaction of academician M.R. Sapin and professor L.E. Etingen: professor G.L. Bilich, professor N.R. Karelina, professor G.S. Katinas, associate professors L.R. Sapozhnikova, O.M. Semyonova, V.G. Kozhukhar and young employees — anatomists, histologists and operative surgeons.

In 1997 Natalia Rafailovna was awarded academic degree of Professor, and from 2000 she headed the Department of Morphology of the Institute of Medical Education.

Natalia Rafailovna devoted a lot of time and energy to creation of the Department of Anatomy museum together with associate professor Oksana Mikhailovna Semyonova. She carried out active methodological work and gave lectures at all faculties of IMO.

The activity of Professor Karelina at that time was not limited to the department: from 1995 to 1997 she is the vice-rector for Science, and from 1997 to 2000 — Dean of the Medical and Dental Faculties.

At that time N.R.Karelina cooperated actively with LPMI departments' staff involving them to deliver lectures and practicals.

In May 2003 she was elected to the position of Head of the Department of Human Anatomy of the St. Petersburg State Pediatric Medical Academys (SPbGPMA) and has headed it successfully for more than 20 years (Fig. 9). Without doubt, all these achievements would have been impossible without the greatest anatomical school that N.R.Karelina went through. She is a successor to anatomical schools of academician M.P. Sapin, V.V. Kupriyanov, and professors G.F. Vsevolodov and M.A. Dolgova. Natalia Rafailovna has a lot of experience in scientific, pedagogical, organizational and methodological work, which definitely helped her in her professional way.

Under N.R. Karelina's leadership, substantial tutorial alterations and additions were made in a lecture course, practicals and examination programme in accordance with new

Fig. 11. II St. Petersburg Symposium on Child Morphology, 2021
Рис. 11. II Санкт-Петербургский симпозиум по морфологии ребенка, 2021 г.

Fig. 12. From left to right: Professor Radik M. Khairullin, professor Natalia R. Karelina, professor Ivan V. Gayvoronsky with 1st year students, 2022
Рис. 12. Слева направо: профессор Радик Магзинурович Хайруллин, профессор Наталья Рафаиловна Карелина, профессор Иван Васильевич Гайворонский со студентами 1-го курса, 2022 г.

anatomical terms. Reorganization of educational process was performed.

Since 2005 studies on the complex theme “Morphological traits of human and experimental animal organism systems in ontogenesis, normal state, experiment and pathology” have been carryed out on the department led by Natalia Rafailovna.

For more than 10 years Natalia Rafailovna was Scientific Secretary of the Dissertation Committee on specialties like “Human Anatomy” and “Cellular biology, cytology, histology”, one of the most respected morphological committees of Russia. Dozens of theses from different regions passed through her hands, which should be carefully reviewed in order to decide whether to admita thesis to defense or to make serious corrections and improvements.

In 2013 Natalia Rafailovna was appointed Dean of the Faculty of Postgraduate and Additional Professional Education of SPbGPMA. During her work as dean N.R. Karelina carried out significant reorganization of the dean’s office changing approaches and methods of its work.

N.R. Karelina is the scientific consultant of two doctoral theses [6, 21] and the scientific supervisor of six candidate (PhD) theses [2–4, 17, 28, 32]. Professor N.R. Karelina is the author of more than 300 scientific works [5, 7–9, 13, 14, 18–20, 22–27, 30, 31, 33, 36–44] including 8 patents for invention, 12 methodical recommendations, 36 tutorial guides [1, 16, 29], 5 textbooks [10, 15, 34, 35] and 3 dictio-

Fig. 13. N.R. Karelina is a young lecturer at the Department of Human Anatomy, 1972

Рис. 13. Н.Р. Карелина — молодой преподаватель кафедры анатомии человека, 1972 г.

naries [1]. She reviewed articles, dissertations, often is an opponent in candidate and doctoral thesis defense, active participant in the activity of the St. Petersburg Branch of the Scientific Medical Society of Anatomists, Histologists and Embryologists (Fig. 10).

Natalia Rafailovna Karelina is deputy editor-in-chief of the journal “Russian Biomedical Research” and a member of editorial board of journals “Morphology”, “Pediatrician”, “Periodontology”, “Forcipe”.

In 2020 N.R. Karelina together with professor R.M. Khayrullin founded and subsequently became president of the St. Petersburg Symposium on Morphology, Biochemistry, Normal and Pathological Physiology of the child, whose goals are to popularize medical science (Fig. 11).

Natalia Rafailovna pays much attention to rising a medical generation, willingly works with students within the framework of a Student Scientific Society, a dissection circle and the “Student-Teacher” project (Fig. 12). It is worth noting that N.R. Karelina headed the Student Scientific Society of the University for a long time. Nowadays as well as in times past she shares her experience, knowledge and wisdom with pleasure and eagerness. At present almost all young lecturers at the department are direct students of professor Karelina.

One cannot help noting Natalia Rafailonva’s refined artistic taste, and it is not without reason, because she was grown up in the family of artists. Apart from this, N.R. Karelina is marked with outstanding willpower, unyieldingness and brilliant organizational abilities, which she owes to her grandfather, Major General Ivan Ivanovich Chezlov. In 1939 he did a 400-kilometer march on the frozen Amur river to the construction site of the city of Komsomolsk-on-Amur without any loss among the soldiers which is a reason for proud in the whole family.

Natalia Rafailovna is a person of everburning energy, optimism and faith in the best, sometimes she can be emotional, strict, but at the same time she is always a very kind and fair, creative, beautiful and charismatic woman (Fig. 13). Whatever happens, she always knows how to help, what to say and what to do. Natalia Rafailovna inspires everybody with her sunny smile and helps to look into the future under new, not always visible angle.

Natalia Rafailovna has got a big and loving family. She is a grandmother, she has got three grandchildren and two great grandchildren, and dozens of devoted disciples for whom she is more than a teacher, who love and appreciate her.

The staff of the Department of Human Anatomy, The University Administration, the Academic Council, the St. Petersburg Branch of the Scientific Medical Society of Anatomists, Histologists and Embryologists, the Editorial Board of the journal “Russian Biomedical Research”, and students cordially congratulate Natalia Rafailovna, wish her good health, inexhaustible energy and creative success for the benefit of her beloved science and her native university.

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ПРАВИЛА ДЛЯ АВТОРОВ

Утв. приказом и.о. ректора
ФГБОУ ВО СПбГПМУ Минздрава России от 23.06.16

НАСТОЯЩИЕ ПРАВИЛА ДЛЯ АВТОРОВ ЯВЛЯЮТСЯ ИЗДАТЕЛЬСКИМ ДОГОВОРОМ

Условия настоящего Договора (далее «Договор») являются публичной офертой в соответствии с п. 2 ст. 437 Гражданского кодекса Российской Федерации. Данный Договор определяет взаимоотношения между редакцией журнала «**Russian Biomedical Research»** (далее по тексту «Журнал»), зарегистрированного Федеральной службой по надзору в сфере связи, информационных технологий и массовых коммуникаций (РОСКОМНАДЗОР), свидетельство: ПИ № ФС77-74228 от 02 ноября 2018 г. (ранее ПИ № ТУ78-01869 от 17 мая 2016 г.), именуемой в дальнейшем «Редакция» и являющейся структурным подразделением ФГБОУ ВО СПбГПМУ Минздрава России, и автором и/или авторским коллективом (или иным правообладателем), именуемым в дальнейшем «Автор», принявшим публичное предложение (оферту) о заключении Договора.

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  Структура основного текста статьи: введение, изложение основного материала, заключение, литература. Для оригинальных исследований — введение, методика, результаты исследования, обсуждение результатов, литература (IMRAD).

  В разделе «методика» обязательно указываются сведения о статистической обработке экспериментального или клинического материала. Единицы измерения даются в соответствии с Международной системой единиц — СИ. Фамилии иностранных авторов, цитируемые в тексте рукописи, приводятся в оригинальной транскрипции.

  Таблицы и рисунки приводятся непосредственно в теле статьи, каждый из которых имеет номер и название с обязательными ссылками на них в тексте статьи — в контексте предложения (например: «…как показано на рисунке 1…») или в конце предложения в круглых скобках (например: «...выявлена положительная корреляционная связь умеренной степени (r=0,41) между уровнем ТТГ матери и новорожденного (рис. 2)»; просьба учитывать, что в печатной версии журнала рисунки будут воспроизводиться в черно-белом варианте.

- Список литературы обязательно в алфавитном порядке: сначала все отечественные, затем иностранные авторы с дополнительным транслитерированным списком (методика транслитерации описана подробно ниже).

Текст статьи должен быть подготовлен в строгом соответствии с настоящими правилами и тщательно выверен автором. В случае обнаружения значительного количества опечаток, небрежностей, пунктуационных и орфографических ошибок, нерасшифрованных сокращений, отсутствия основных компонентов и других технических дефектов оформления статей редакция возвращает статью автору для доработки. Небольшие погрешности редакция может исправить сама без согласования с автором. Кроме того, редакция оставляет за собой право осуществления литературного редактирования статей.

Сокращений, кроме общеупотребляемых, следует избегать. Сокращения в названии статьи, названиях таблиц и рисунков, в выводах недопустимы. Если аббревиатуры используются, то все они должны быть непременно расшифрованы полностью при первом их упоминании в тексте (например: «Наряду с данными о РОН (резидуально-органической недостаточности), обусловливающей развитие ГКС (гиперкинетического синдрома), расширен диапазон исследований по эндогенной природе данного синдрома».

Все цитирования производятся следующим образом:

ФИО автора, год издания и прочая информация не упоминаются в тексте. Вместо этого указывается ссылка на источник литературы в виде номера в квадратных скобках (пример: «Ряд исследователей отмечает различные нарушения речевых функций при эпилепсии в детском возрасте [17, 21, 22].»), который включен в расставленный в алфавитном порядке список источников в конце статьи.

Все ссылки должны иметь соответствующий источник в списке, а каждый источник в списке — ссылку в тексте.

В виде исключения в тексте могут приводиться ФИО конкретных авторов в формате И. О. Фамилия, год и даже название источника, но при этом все равно обязательна ссылка (в квадратных скобках в конце предложения) на источник, включенный в список литературы.

(Например: «В 1892 году великий Эраст Гамильтонский описал в своем бессмертном труде «Об открытии третьего уха у человека» третье (непарное) ухо» [34].)

Литература (References)

Учитывая требования международных систем цитирования, список литературы приводится не только в обычном виде, но также и дополнительно в транслитерированном (см. Транслитерация).

В статье приводятся ссылки на все упоминаемые в тексте источники.

Фамилии и инициалы авторов в пристатейном списке приводятся в алфавитном порядке, сначала русского, затем латинского алфавита.

В описании указываются все авторы публикации.

Библиографические ссылки в тексте статьи даются в квадратных скобках.

Ссылки на неопубликованные работы не допускаются.

Список литературы комплектуется в следующем порядке:

Нормативные акты

Приказы, нормативные акты, методические письма и прочие законные акты, патенты, полезные модели не вносятся в список литературы, оформляются в виде сносок. Сноска — примечание, помещаемое внизу страницы (постраничная сноска). Знак сноски ставят цифрой после фрагмента основного текста, где есть упоминание об этих источниках. Рекомендуется сквозная нумерация сносок по тексту.

Интернет-ресурс

1. Интернет-ресурс, где есть название источника, автор — вносится в список литературы (в порядке алфавита) с указанием даты обращения (см. ниже пример оформления).

2. Если есть только ссылка на сайт — вносится в список литературы в конце, с указанием даты обращения.

Щеглов И. Насколько велика роль микрофлоры в биологии вида-хозяина? Живые системы: научный электронный журнал. Доступен по: http://www.biorf.ru/catalog.aspx?cat_id=396&d_no=3576 (дата обращения 02.07.2012).

Kealy M. A., Small R. E., Liamputtong P. Recovery after caesarean birth: a qualitative study of women's accounts in Victoria, Australia. BMC Pregnancy and Childbirth. 2010. Available at: http://www.biomedcentral. com/1471–2393/10/47/ (Accessed 11.09.2013).

Книга

Автор(ы) название книги (знак точка) место издания (двоеточие) название издательства (знак точка с запятой) год издания. Если в качестве автора книги выступает редактор, то после фамилии следует ред.

Айламазян Э.К., Новиков Б.Н., Зайнулина М.С., Палинка Г.К., Рябцева И.Т., Тарасова М.А. Акушерство: учебник. 6-е изд. СПб.; 2007.

Преображенский Б.С., Темкин Я.С., Лихачев А.Г. Болезни уха, горла и носа. М.: Медицина; 1968.

Радзинский В.Е., ред. Перинеология: учебное пособие. М.: РУДН; 2008.

Brandenburg J.H., Ponti G.S., Worring A.F. eds. Vocal cord injection with autogenous fat. 3 rd ed. NY:Mosby; 1998.

Domeika M. Diagnosis of genital chlamydial infection in humans as well as in cattle. Uppsala; 1994.

Глава из книги

Автор(ы) название главы (знак точка) В кн.: или In: далее описание книги [Автор(ы) название книги (знак точка) место издания (двоеточие) название издательства (знак точка с запятой) год издания] (двоеточие) стр. от и до.

Коробков Г.А. Темп речи. В кн.: Современные проблемы физиологии и патологии речи: сб. тр. Т. 23. М.; 1989: 107–11.

Статья из журнала:

Автор(ы) название статьи (знак точка) название журнала (знак точка) год издания (знак точка с запятой) том (если есть в круглых скобках номер журнала) затем знак (двоеточие) страницы от и до.

Кирющенков А.П., Совчи М.Г., Иванова П.С. Поликистозные яичники. Акушерство и гинекология. 1994; N 1: 11–4.

Brandenburg J.H., Ponti G.S., Worring A.F. Vocal cord injection with autogenous fat: a long-term magnetic resona. Laryngoscope. 1996; l06(2,pt l): 174–80.

Simpson J. et al. Association between adverse perinatal outcomes and serially obtained second and third trimester MS AFP measurements. Am. J. Obstet. Gynecol. 1995; 173: 1742.

Deb S., Campbell B.K., Pincott-Allen C. et al. Quantifying effect of combined oral contraceptive pill on functional ovarian reserve as measured by serum anti-Müllerian hormone and small antral follicle count using three-dimensional ultrasound. Ultrasound. Obstet. Gynecol. 2012; 39 (5): 574–80.

Тезисы докладов, материалы научных конференций

Бабий А.И., Левашов М.М. Новый алгоритм нахождения кульминации экспериментального нистагма (миниметрия). III съезд оториноларинг. Беларуси: тез. докл. Минск; 1992: 68–70.

Салов И.А., Маринушкин Д.Н. Акушерская тактика при внутриутробной гибели плода. В кн.: Материалы IV Российского форума «Мать и дитя». М.; 2000; ч. 1: 516–9.

Авторефераты

Петров С.М. Время реакции и слуховая адаптация в норме и при периферических поражениях слуха. Автореф. дис. … канд. мед. наук. СПб.; 1993.

Прочее

World Health Organization. Prevalence and incidence of selected sexually transmitted infections, 2005 global estimates. Geneva: World Health Organization; 2011.

Транслитерация

Список литературы подается в двух вариантах: первый на языке оригинала (русскоязычные источники кириллицей, англоязычные латиницей), второй — (References) в романском алфавите (для Scopus и других международных баз данных, повторяя в нем все источники литературы, независимо от того, имеются ли среди них иностранные). Если в списке есть ссылки на иностранные публикации, они полностью повторяются в списке, готовящемся в романском алфавите.

В романском алфавите для русскоязычных источников требуется следующая структура библиографической ссылки: автор(ы) (транслитерация), [перевод названия книги или статьи на английский язык], название источника (транслитерация), выходные данные в цифровом формате, указание на язык статьи в скобках (in Russian).

Пример:

Preobrazhenskiy B.S., Temkin Ya. S., Likhachev A.G. Bolezni ukha, gorla i nosa [Diseases of the ear, nose and throat]. M.: Meditsina; 1968. (in Russian).

Технология подготовки ссылок с использованием системы автоматической транслитерации и переводчика:

На сайте http://www.translit.ru можно бесплатно воспользоваться программой транслитерации русского текста в латиницу. Программа очень простая.

Входим в программу Translit.ru. В окошке «варианты» выбираем систему транслитерации BGN (Board of Geographic Names). Вставляем в специальное поле весь текст библиографии на русском языке и нажимаем кнопку «в транслит».

Копируем транслитерированный текст в готовящийся список References. Переводим на английский язык название книги, статьи, постановления и т.д., переносим его в готовящийся список. **Внимание!** Необходим авторский корректный перевод названия. Автоматический перевод, предполагающий возможное искажение сути названия статьи, недопустим.

Объединяем описания в соответствии с принятыми правилами и редактируем список. В конце ссылки в круглых скобках указывается (in Russian). Ссылка готова.

Примеры транслитерации русскоязычных источников литературы для англоязычного блока статьи.

Книга: Avtor (y) Nazvanie knigi (znak tochka) [The title of the book in english]. mesto izdaniya (dvoetochie) nazvanie izdatel'stva (znak tochka s zapyatoy) god izdaniya.

Preobrazhenskiy B.S., Temkin Ya.S., Likhachev A.G. Bolezni ukha, gorla i nosa [Diseases of the ear, nose and throat]. M.: Meditsina; 1968. (in Russian).

Radzinskiy V. E., ed. Perioneologiya: uchebnoe posobie [Perineology tutorial]. M.: RUDN; 2008. (in Russian).

Глава из книги: Avtor (y) nazvanie glavy (znak tochka) [The title of the article in english]. In: Avtor (y) nazvanie knigi (znak tochka) mesto izdaniya (dvoetochie) nazvanie izdatel'stva (znak tochka s zapyatoy) god izdaniya]. (dvoetochie) str. ot i do.

Korobkov G. A. Temp rechi [Rate of speech]. V kn.: Sovremennye problemy fiziologii i patologii rechi: sb. tr. T. 23. M.;1989:107–11. (in Russian).

Статья из журнала: Avtor (y) nazvanie stat'i [The title of the article in english] (znak tochka) nazvanie zhurnala (znak tochka) god izdaniya (znak tochka s zapyatoy) tom (esli est' v kruglykh skobkakh nomer zhurnala) zatem znak (dvoetochie) stranitsy ot i do.

Kiryushchenkov A. P., Sovchi M. G., Ivanova P. S. Polikistoznye yaichniki [Polycystic ovary]. Akusherstvo i ginekologiya. 1994; N 1: 11–4. (in Russian).

Тезисы докладов, материалы научных конференций

Babiy A. I., Levashov M. M. Novyy algoritm nakhozhdeniya kul'minatsii eksperimental'nogo nistagma (minimetriya) [New algorithm of finding of the culmination experimental nystagmus (minimetriya)]. III s»ezd otorinolaringologov Resp. Belarus': tez. dokl. Minsk; 1992: 68–70. (in Russian).

Salov I. A., Marinushkin D. N. Akusherskaya taktika pri vnutriutrobnoy gibeli ploda [Obstetric tactics in intrauterine fetal death]. V kn.: Materialy IV Rossiyskogo foruma «Mat' i ditya». M.; 2000; ch.1:516–9. (in Russian).

Авторефераты

Petrov S. M. Vremya reaktsii i slukhovaya adaptatsiya v norme i pri perifericheskikh porazheniyakh slukha [Time of reaction and acoustical adaptation in norm and at peripheral defeats of hearing]. PhD thesis. SPb.; 1993. (in Russian).

Описание Интернет-ресурса

Shcheglov I. Naskol'ko velika rol' mikroflory v biologii vida-khozyaina? [How great is the microflora role in type-owner biology?]. Zhivye sistemy: nauchnyy elektronnyy zhurnal. Available at: http://www.biorf.ru/catalog.aspx?cat_id=396&d_no=3576 (accessed 02.07.2012). (in Russian).

Пример списка литературы, включающего транслитерированный вариант:

ЛИТЕРАТУРА

1. Кофиади И.А. Генетическая устойчивость к заражению ВИЧ и развитию СПИД в популяциях России и сопредельных государств. Автореф. дис. ... канд. биол. наук. М.; 2008. Доступен по: http://www.dnatechnology.ru/ files/ images/d/0b136b567d25d4be1dfa26a8b39ec2b9.pdf (дата обращения 18.09.2014).
2. Flynn E., Eyre S., Packham J. Childhood Arthritis Prospective Study (CAPS), UKRAG Consortium, BSPAR Study Group, Barton A., Worthington J., Thomson W. Association of the CCR5 gene with juvenile idiopathic arthritis. Genes Immun. 2010; 11 (7): 584–89.

и т.д.

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1. Kofiadi I.A. Geneticheskaya stoychivost' k zarazheniyu VICh i razvitiyu SPID v populyatsiyakh Rossii i sopredel'nykh gosudarstv [Genetic resistance to HIV infection and development of AIDS in populations of Russia and neighboring countries]. PhD-thesis. M.; 2008. Available from: http://www.dna-technology.ru/files/images/d/0b136b567d25d4be1dfa26a8b39ec2b9.pdf (accessed 18.09.2014) (in Russian).
2. Flynn E., Eyre S., Packham J. Childhood Arthritis Prospective Study (CAPS), UKRAG Consortium, BSPAR Study Group, Barton A., Worthington J., Thomson W. Association of the CCR5 gene with juvenile idiopathic arthritis. Genes Immun. 2010; 11 (7): 584–89.

Etc.

Для всех статей, имеющих DOI, индекс необходимо указывать в конце библиографического описания.

ОТВЕТСТВЕННОСТЬ ЗА ПРАВИЛЬНОСТЬ БИБЛИОГРАФИЧЕСКИХ ДАННЫХ НЕСЕТ АВТОР.

АВТОРСКОЕ ПРАВО

Редакция отбирает, готовит к публикации и публикует переданные Авторами материалы. Авторское право на конкретную статью принадлежит авторам статьи. Авторский гонорар за публикации статей в Журнале не выплачивается. Автор передает, а Редакция принимает авторские материалы на следующих условиях:

1) Редакции передается право на оформление, издание, передачу Журнала с опубликованным материалом Автора для целей реферирования статей из него в Реферативном журнале ВИНИТИ, РНИЦ и базах данных, распространение Журнала/авторских материалов в печатных и электронных изданиях, включая размещение на выбранных либо созданных Редакцией сайтах в сети Интернет в целях доступа к публикации в интерактивном режиме любого заинтересованного лица из любого места и в любое время, а также на распространение Журнала с опубликованным материалом Автора по подписке;
2) территория, на которой разрешается использовать авторский материал, — Российская Федерация и сеть Интернет;
3) срок действия Договора — 5 лет. По истечении указанного срока Редакция оставляет за собой, а Автор подтверждает бессрочное право Редакции на продолжение размещения авторского материала в сети Интернет;
4) Редакция вправе по своему усмотрению без каких-либо согласований с Автором заключать договоры и соглашения с третьими лицами, направленные на дополнительные меры по защите авторских и издательских прав;
5) Автор гарантирует, что использование Редакцией предоставленного им по настоящему Договору авторского материала не нарушит прав третьих лиц;
6) Автор оставляет за собой право использовать предоставленный по настоящему Договору авторский материал самостоятельно, передавать права на него по договору третьим лицам, если это не противоречит настоящему Договору;
7) Редакция предоставляет Автору возможность безвозмездного получения справки с электронными адресами его официальной публикации в сети Интернет;
8) при перепечатке статьи или ее части ссылка на первую публикацию в Журнале обязательна.

ПОРЯДОК ЗАКЛЮЧЕНИЯ ДОГОВОРА

Заключением Договора со стороны Редакции является опубликование рукописи данного Автора в журнале «Russian Biomedical Research» и размещение его текста в сети Интернет. Заключением Договора со стороны Автора, т.е. полным и безоговорочным принятием Автором условий Договора, является передача Автором рукописи и экспертного заключения.

РЕЦЕНЗИРОВАНИЕ

Статьи, поступившие в редакцию, обязательно рецензируются. Если у рецензента возникают вопросы, то статья с комментариями рецензента возвращается Автору. Датой поступления статьи считается дата получения Редакцией окончательного варианта статьи. Редакция оставляет за собой право внесения редакторских изменений в текст, не искажающих смысла статьи (литературная и технологическая правка).

АВТОРСКИЕ ЭКЗЕМПЛЯРЫ ЖУРНАЛА

Редакция обязуется выдать Автору 1 экземпляр Журнала на каждую опубликованную статью вне зависимости от числа авторов. Авторы, проживающие в Санкт-Петербурге, получают авторский экземпляр Журнала непосредственно в Редакции. Иногородним Авторам авторский экземпляр Журнала высылается на адрес автора по запросу от автора. Экземпляры спецвыпусков не отправляются авторам.

АДРЕС РЕДАКЦИИ

194100, Санкт-Петербург, Литовская ул., 2
e-mail: scrcenter@mail.ru. Сайт журнала: http://www.gpmu.org/science/pediatrics-magazine/Russian_Biomedical_Research.