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ЕЖЕМЕСЯЧНЫЙ НАУЧНЫЙ ЖУРНАЛ

Медицинские новости Грузии

საქართველოს სამედიცინო სიახლენი

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გამოიცემა თბილისის სახელმწიფო სამედიცინო უნივერსიტეტთან
თანამშრომლობითა და მისი პატრონაჟით

ЕЖЕМЕСЯЧНЫЙ НАУЧНЫЙ ЖУРНАЛ

ТБИЛИСИ - НЬЮ-ЙОРК

GMN: Georgian Medical News is peer-reviewed, published monthly journal committed to promoting the science and art of medicine and the betterment of public health, published by the GMN Editorial Board and The International Academy of Sciences, Education, Industry and Arts (U.S.A.) since 1994. **GMN** carries original scientific articles on medicine, biology and pharmacy, which are of experimental, theoretical and practical character; publishes original research, reviews, commentaries, editorials, essays, medical news, and correspondence in English and Russian.

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GMN: Медицинские новости Грузии - ежемесячный рецензируемый научный журнал, издаётся Редакционной коллегией и Международной академией наук, образования, искусств и естествознания (IASEIA) США с 1994 года на русском и английском языках в целях поддержки медицинской науки и улучшения здравоохранения. В журнале публикуются оригинальные научные статьи в области медицины, биологии и фармации, статьи обзорного характера, научные сообщения, новости медицины и здравоохранения.

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GMN: Georgian Medical News – საქართველოს სამედიცინო სიახლენი – არის ყოველთვიური სამეცნიერო სამედიცინო რეცენზირებადი ჟურნალი, გამოიცემა 1994 წლიდან, წარმოადგენს სარედაქციო კოლეგიისა და აშშ-ის მეცნიერების, განათლების, ინდუსტრიის, ხელოვნებისა და ბუნებისმეტყველების საერთაშორისო აკადემიის ერთობლივ გამოცემას. GMN-ში რუსულ და ინგლისურ ენებზე ქვეყნდება ექსპერიმენტული, თეორიული და პრაქტიკული ხასიათის ორიგინალური სამეცნიერო სტატიები მედიცინის, ბიოლოგიისა და ფარმაციის სფეროში, მიმოხილვითი ხასიათის სტატიები.

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При представлении в печать научных экспериментальных работ авторы должны указывать вид и количество экспериментальных животных, применявшиеся методы обезболивания и усыпления (в ходе острых опытов).

4. К статье должны быть приложены краткое (на полстраницы) резюме на английском, русском и грузинском языках (включающее следующие разделы: цель исследования, материал и методы, результаты и заключение) и список ключевых слов (key words).

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2. Size of the article, including index and resume in English, Russian and Georgian languages must be at least 10 pages and not exceed the limit of 20 pages of typed or computer-printed text.

3. Submitted material must include a coverage of a topical subject, research methods, results, and review.

Authors of the scientific-research works must indicate the number of experimental biological species drawn in, list the employed methods of anesthetization and soporific means used during acute tests.

4. Articles must have a short (half page) abstract in English, Russian and Georgian (including the following sections: aim of study, material and methods, results and conclusions) and a list of key words.

5. Tables must be presented in an original typed or computer-printed form, instead of a photocopied version. **Numbers, totals, percentile data on the tables must coincide with those in the texts of the articles.** Tables and graphs must be headed.

6. Photographs are required to be contrasted and must be submitted with doubles. Please number each photograph with a pencil on its back, indicate author's name, title of the article (short version), and mark out its top and bottom parts. Drawings must be accurate, drafts and diagrams drawn in Indian ink (or black ink). Photocopies of the X-ray photographs must be presented in a positive image in **tiff format**.

Accurately numbered subtitles for each illustration must be listed on a separate sheet of paper. In the subtitles for the microphotographs please indicate the ocular and objective lens magnification power, method of coloring or impregnation of the microscopic sections (preparations).

7. Please indicate last names, first and middle initials of the native authors, present names and initials of the foreign authors in the transcription of the original language, enclose in parenthesis corresponding number under which the author is listed in the reference materials.

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In GMN style for each work cited in the text, a bibliographic reference is given, and this is located at the end of the article under the title "References". All references cited in the text must be listed. The list of references should be arranged alphabetically and then numbered. References are numbered in the text [numbers in square brackets] and in the reference list and numbers are repeated throughout the text as needed. The bibliographic description is given in the language of publication (citations in Georgian script are followed by Cyrillic and Latin).

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ავტორთა საყურადღებოდ!

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1. სტატია უნდა წარმოადგინოთ 2 ცალად, რუსულ ან ინგლისურ ენებზე,დაბეჭდილი სტანდარტული ფურცლის 1 გვერდზე, 3 სმ სიგანის მარცხენა ველისა და სტრიქონებს შორის 1,5 ინტერვალის დაცვით. გამოყენებული კომპიუტერული შრიფტი რუსულ და ინგლისურენოვან ტექსტებში - **Times New Roman (Кириллица)**, ხოლო ქართულენოვან ტექსტში საჭიროა გამოვიყენოთ **AcadNusx.** შრიფტის ზომა – 12. სტატიას თან უნდა ახლდეს CD სტატიით.

2. სტატიის მოცულობა არ უნდა შეადგენდეს 10 გვერდზე ნაკლებს და 20 გვერდზე მეტს ლიტერატურის სიის და რეზიუმეების (ინგლისურ, რუსულ და ქართულ ენებზე) ჩათვლით.

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6. ფოტოსურათები უნდა იყოს კონტრასტული; სურათები, ნახაზები, დიაგრამები - დასათაურებული, დანომრილი და სათანადო ადგილას ჩასმული. რენტგენოგრამების ფოტოასლები წარმოადგინეთ პოზიტიური გამოსახულებით **tiff** ფორმატში. მიკროფოტოსურათების წარწერებში საჭიროა მიუთითოთ ოკულარის ან ობიექტივის საშუალებით გადიდების ხარისხი, ანათალების შეღებვის ან იმპრეგნაციის მეთოდი და აღნიშნოთ სურათის ზედა და ქვედა ნაწილები.

7. სამამულო ავტორების გვარები სტატიაში აღინიშნება ინიციალების თანდართვით, უცხოურისა – უცხოური ტრანსკრიპციით.

8. სტატიას თან უნდა ახლდეს ავტორის მიერ გამოყენებული სამამულო და უცხოური შრომების ბიბლიოგრაფიული სია (ბოლო 5-8 წლის სიღრმით). ანბანური წყობით წარმოდგენილ ბიბლიოგრაფიულ სიაში მიუთითეთ ჯერ სამამულო, შემდეგ უცხოელი ავტორები (გვარი, ინიციალები, სტატიის სათაური, ჟურნალის დასახელება, გამოცემის ადგილი, წელი, ჟურნალის №, პირველი და ბოლო გვერდები). მონოგრაფიის შემთხვევაში მიუთითეთ გამოცემის წელი, ადგილი და გვერდების საერთო რაოდენობა. ტექსტში კვადრატულ ფჩხილებში უნდა მიუთითოთ ავტორის შესაბამისი N ლიტერატურის სიის მიხედვით. მიზანშეწონილია, რომ ციტირებული წყაროების უმეტესი ნაწილი იყოს 5-6 წლის სიღრმის.

9. სტატიას თან უნდა ახლდეს: ა) დაწესებულების ან სამეცნიერო ხელმძღვანელის წარდგინება, დამოწმებული ხელმოწერითა და ბეჭდით; ბ) დარგის სპეციალისტის დამოწმებული რეცენზია, რომელშიც მითითებული იქნება საკითხის აქტუალობა, მასალის საკმაობა, მეთოდის სანდოობა, შედეგების სამეცნიერო-პრაქტიკული მნიშვნელობა.

10. სტატიის ბოლოს საჭიროა ყველა ავტორის ხელმოწერა, რომელთა რაოდენობა არ უნდა აღემატებოდეს 5-ს.

11. რედაქცია იტოვებს უფლებას შეასწოროს სტატია. ტექსტზე მუშაობა და შეჯერება ხდება საავტორო ორიგინალის მიხედვით.

12. დაუშვებელია რედაქციაში ისეთი სტატიის წარდგენა, რომელიც დასაბეჭდად წარდგენილი იყო სხვა რედაქციაში ან გამოქვეყნებული იყო სხვა გამოცემებში.

აღნიშნული წესების დარღვევის შემთხვევაში სტატიები არ განიხილება.

Содержание:

შედარებით. განსხვავება სხეულის მასის შემცირებასა ან ნახშირწყლების ცვლის მაჩვენებლების გაუმჯობესებაში არ აღინიშნა.

CGM-ის გამოყენება პაციენტებში გიდ-ით დაკავშირებულია სიცოცხლის ხარისხის გაუმჯობესებასთან და კმაყოფილების უფრო მაღალ ხარისხთან, HbA1c-ის და უზმოდ გლუკოზის დონის შემცირებასთან, ფიზიკური დატვირთვების დონის ზრდასთან.

VARIANTS OF *IL1* (C3954T, RS1143634), *PON1* (C108T, RS705379) GENES AS PROGNOSTIC MARKERS OF OSTEOMYELITIS RISK AND ITS COMPLICATIONS

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Number of surgeries using implants constantly increases. Most orthopedic and traumatological surgeries use various fixators for osteosynthesis, which allows to achieve bone union and restore fitness. A very serious problem that can arise for the patient and the surgeon after surgery is infectious complications. According to different authors, infection after osteosynthesis is in range from 3.6% to 8.1%, while for open multi-fractured bone traumas may reach 30.0% [2]. There are many proven risk factors, which significantly influence on the risk of septic process in the area of an implant; those are: systemic diseases (diabetes, cancer), bad habits (smoking, drug use), effects of some medications, severity of injury, experience of the doctor, the level of aseptic hospital preparation and more [2,15,18,23]. But quite often we encounter cases when, after a perfectly performed osteosynthesis in a healthy young patient, after a simple closed fracture, there is suppuration of the postoperative wound. The result of infectious complications after osteosynthesis is an increase of the treatment duration and, consequently, its cost. The development of chronic osteomyelitis (M 86 according to the International Classification of Diseases), a disease, in which the infection affects all bone structure, with the development of sepsis and even fatalities in 1-2% of patients, worsens the healing process and quality of life of patients [22].

That is why we began studying possible genetic preconditions for the infection development after osteosynthesis. Genetic factors as diagnostic markers and markers of risk are studied for both nonbacterial [6] and bacterial osteomyelitis [24]. *LPIN2, PSTPIP2* and *IL1RN* genes, which cause IL-1-mediated inflammation, have been shown to play an important role in the development of chronic recurrent multifocal osteomyelitis, and *LPIN2* deficiency may activate the *NLRP3* gene and increase inflammatory response without bacterial pathogens. Given this and the results of the full-genome sequencing, the authors confirmed that nonbacterial osteomyelitis is a complex genetic disorder [6]. But, in our opinion, it is likely that greater role is played by genetic predisposition, when under the influence of exogenous factors (presence/absence of bacterial pathogens, metabolic disorders, traumas, environment pollution), in carriers of gene variants, and, especially, cytokine genes, risk of excessive inflammatory reaction is increased. These results were obtained in separate studies, where it was directly emphasized that there are population differences in the frequency of distribution of gene variants that control cytokines production [3,10]. However, similar studies on the effect of gene variants on the risk of recurrent bacterial osteomyelitis have not received sufficient attention in various population samples.

We can expect that new methods of molecular genetic diagnosis will help to identify rare genetic syndromes with similar clinical features, inheritable by descendants [6]. But the genes responsible for the cytokines production will in any case have a modifying effect, especially in the deepening of metabolic disorders due to concomitant pathology. Therefore, when selecting candidate genes for the study, we chose a variant of the *IL1B* gene (C3954T, rs1143634), a single nucleotide substitution in which leads to a change in the level of its expression. The main clinically significant option is, according to previous studies, the replacement of cytosine (C) by thymine (T) at position 3954, resulting in increased cytokine synthesis. In heterozygous individuals it increases approximately twice, and in homozygous with 3954TT genotype – 4 times, in comparison with homozygous for the common 3954CC genotype. That is, inflammatory processes in carriers of polymorphic variants of the *IL1B* gene occur more actively. In homozygous carriers of 3954TT variant there is an increased risk for gaining excess weight and developing metabolic syndrome [4], the manifestations of which are known to complicate recovery from osteomyelitis [5,18].

Taking into account stated above, as a second gene candidate *PON1* (C108T, rs705379) was chosen, in which replacement of cytosine (C) to thymine (T) at position 108 promoter regions is associated with decreased gene expression, resulting in a reduction of protection lipoprotein low density from oxidative modification under oxidative stress and the impact of a polluted environment, which provokes the development of progressive metabolic disorders and atherosclerotic vascular lesions, but medicine correction can reduce the risk of complications due to increased gene expression [14]. Therefore, genetic testing and analysis of gene variants is an important basis for identifying individuals at risk of developing bacterial osteomyelitis, its complicated course and choosing a personalized strategy for its prevention and treatment.

Purpose of the work - to study the effect of *IL1B* (C3954T, rs1143634), *PON1* (C108T, rs705379) gene variants on the risk of bacterial osteomyelitis development and its complicated course.

Material and methods. For our study, we selected a group of 56 patients who were diagnosed with a bacterial infection in the area of surgery, or traumatic osteomyelitis of the long bones of the extremities after osteosynthesis due to traumatic fractures. Patients involved in the study were treated in the department of bone-purulent surgery of SI "Institute of Traumatology and Orthopedics of NAMS of Ukraine" from 2011 to 2020. In the vast majority of patients, a microbial agent was detected – 87.5%, in 75.5% cases gram-positive flora (in 13.5% of these cases – resistant).

The average age of patients was 43.1±14.6 years. Among patients there was predominantly men – 39 (69.6%) and 17 women (30.4%). On-bone plates for fixation of fragments were used in 37 (67.2 %) patients and locking intramedullary rods in18 (37.8%) patients. Infection in the area of surgical intervention developed at different times after surgery – the average term was 1.5 years. We divided patients in groups for the study, according to the course of the infectious process – non-recurrent and recurrent osteomyelitis. 20 (35.7 %) patients had uncomplicated (non-recurrent) course after surgical osteomyelitis treatment, recovered and at remote observation had no recurrence of infectious process. Accordingly, complicated (recurrent), ineffective treatment with removal of metal construction and with recurrent infection were observed in 36 (64.3%) patients. As a control group, population frequency data for persons of European population were used, which were taken from the open database of the project "1000 Genomes" [1]. A permission to conduct research and write the publication was obtained from the Ethics Committee of the of SI "Institute of Traumatology and Orthopedics of NAMS of Ukraine".

For molecular genetic analysis, DNA was isolated from peripheral blood samples using the Quick-DNA Mini Prep Plus Kit (Zymo Research, USA) according to the instructions. *PON1* (C108T, rs705379) and *IL1B* (C3954T, rs1143634) gene variants studying was performed according to the modified protocols using the polymerase chain reaction (PCR) and method of restriction fragment length polymorphism (PCR-RFLP) [8, 19].

DNA fragments of *PON1* and *IL1B* genes were amplified using a commercial DreamTaq Green PCR Master Mix (Thermo Scientific, USA) and specific oligonucleotide primers (Metabion, Germany) using standard polymerase chain reaction techniques. The amplification products were hydrolyzed by restriction endonucleases (Thermo Scientific, USA). Information about the primers sequence and restriction endonucleases is presented in Table 1.

The digested products were separated using agarose gel electrophoresis and visualized on a UV transilluminator (Figs. 1, 2).

Table 1. Summary of PCR-RFLP analysis

Gene variant	Primers sequence (5′ to 3′)	Annealing t (^{0}C)	Restriction enzyme	Size of amplicon and restriction fragments (bp)
PON1 C108T	GGCTGCAGCCCTCACCACAACCC	68	MbiI	Amplicon : 240 108C: 28*, 212 108T: 240
	AGCTAGCTGCCGACCCGGCGGGG			
IL1B C3954T	TTCAGTTCATATGGACCAGA	54	TaqI	Amplicon : 249 3954C : 114, 135 3954T : 249
	GTTGTCATCAGACTTTGACC			

** - fragments up to 30 base pairs (bp) in agarose gel were not visualized*

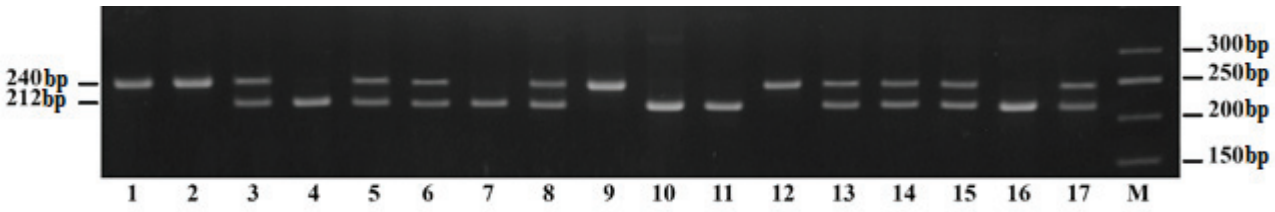

Fig. 1. Electrophoregram of restriction fragments for the C108T variant of the PON1 gene: 1, 2, 9, 12 – 108TT genotype; 3, 5, 6, 8, 13-15, 17 – 108CT genotype; 4, 7, 10, 11, 16 – 108CC genotype; M – DNA marker

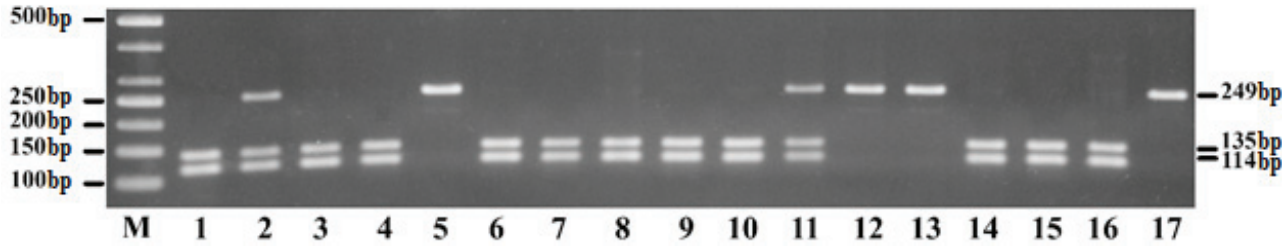

Fig. 2. Electrophoregram of restriction fragments for the C3954T variant of the IL1B gene: M – DNA marker; 1, 3, 4, 6-10, 14-16 – 3954CC genotype; 2, 11 – 3954CT genotype; 5, 12, 13, 17 – 3954TT genotype

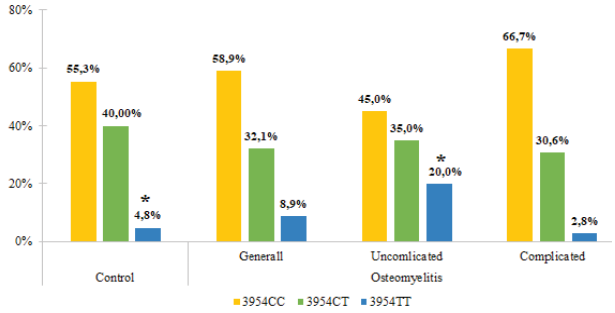

*Fig. 3. The frequency of the IL1B (C3954T) gene variants distribution in the comparison groups. Note: * – significant difference in genotype frequencies was revealed*

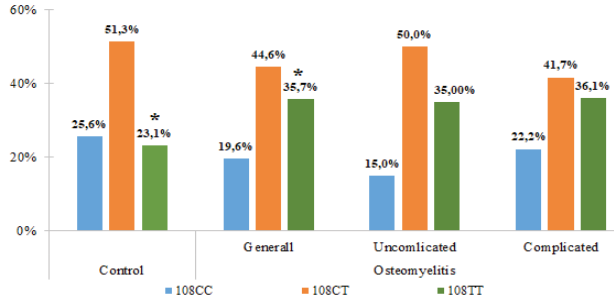

*Fig. 4. The frequency of the PON1 (C108T) gene variants distribution in the comparison groups. Note: * - significant difference in genotype frequencies was revealed*

Table 2. Distribution of the IL1B (C3954T), PON1 (C108T) genes variants in comparison subgroups depending on gender

Genes variants		Control group	Osteomyelitis (generall)	Uncompl. subgroup	Compl. subgroup	Statistical differences
Male						
PON1 C108T	108CC	60 (25.0%)	8 (20.5%)	1 (8.3%)	7 (25.9%)	p>0.05
	108CT	122 (50.8%)	18 (46.2%)	7 (58.3%)	11 (40.7%)	p>0.05
	108TT	58 (24.2%)	13 (33.3%)	4 (33.4%)	9 (33.3%)	p>0.05
IL1B C3954T	3954CC	128 (53.3%)	28* (71.8%)	7 (58.3%)	21** (77.8%)	*χ^2=4.64, p=0.031, OR=2.23 (1.06-4.68) **χ^2=4.93, p=0.026, OR=3.06 (1.19-7.86)
	3954CT	97 (40.4%)	8* (20.5%)	3 (25.0%)	5** (18.5%)	*χ^2=4.85, p=0.028, OR=0.38 (0.17-0.86) **χ^2=4.05, p=0.044, OR=0.34 (0.12-0.92)
	3954TT	15 (6.2%)	3 (7.7%)	2 (16.7%)	1 (3.7%)	p>0.05
Female						
PON1 C108T	108CC	69 (26.2%)	3 (17.6%)	2 (25.0%)	1 (11.1%)	p>0.05
	108CT	136 (51.7%)	7 (41.2%)	3 (37.5%)	4 (44.4%)	p>0.05
	108TT	58 (22.1%)	7 (41.2%)	3 (37.5%)	4 (44.4%)	p>0.05
IL1B C3954T	3954CC	150 (57.0%)	5 (29.4%)	2 (25.0%)	3 (33.3%)	p>0.05
	3954CT	104 (39.5%)	10 (58.8%)	4 (50.0%)	6 (66.7%)	p>0.05
	3954TT	9 (3.4%)	2 (11.8%)	2* (25.0%)	0 (0.0%)	*χ^2=4.57, p=0.026, OR=9.41 (1.66-53.22)

*note: *, ** – compared to the control group*

Statistical processing of data was performed used Microsoft Excel Pro Plus 2016 and SPSS v.27. Genotype and allele frequencies in case and control groups were analyzed using the χ^2 test. The association of variants of the studied genes with the risk of developing bacterial osteomyelitis and its complicated course was investigated by calculating the odds ratio (OR) within 95% of the confidence interval (CI). Differences were considered significant for all types of analysis at a significance level (p) of less than 0.05.

Results and discussion. To assess the effect of *IL1B* and *PON1* gene variants, we compared the frequencies in the general group of patients with osteomyelitis and subgroups, depending on its course, with the frequencies in the control group (Fig.3 and Fig.4, respectively).

The frequency of genotypes distribution of *IL1B* (C3954T) gene did not differ significantly in the general group of patients compared with the control group. In patients with uncomplicated course of the disease there was significantly increased distri-

bution frequency of 3954TT genotype compared to the control group (χ^2=6.05, p=0.014, OR=4.99 (1.55-16.07)). The presence of this genotype almost 5 times increased the likelihood of uncomplicated osteomyelitis in patients.

During *PON1* (C108T) gene variants analysis there were found significant increased incidence of 108TT genotype in patients with osteomyelitis compared to patients of control group (χ^2=4.38, p=0.036, OR=1.85(1.03-3.33)). But the frequency of *PON1* gene variants distribution did not differ when compared patients with osteomyelitis depending on complications.

Among the examined patients, men were predominated, which is known characteristic of complicated osteomyelitis [12]. Therefore, taking into account gender differences, we conducted additional statistical analysis for male and female patients separately (Table 2).

The frequency of osteomyelitis complications in male patients was increased – 69.23% compared to female patients – 52.94%, but these indicators did not differ significantly. At the same time, men with osteomyelitis compared to men in the control group had a significantly reduced frequency of 3954CT genotype and a significantly increased frequency of 3954CC genotype in the *IL1B* gene. It is obvious that for men, the presence even one 3954T allele was a protective against the development of osteomyelitis, and the absence of this allele – genotype 3954CC was a risk factor for complications in the treated patients with osteomyelitis. And among female patients with an uncomplicated course of the disease, we found a significant increase in the prevalence of 3954TT genotype in contrast to women in the control group. According to the variants of the *PON1* gene, we did not find any significant differences between genotypes frequencies in the comparison groups that took into account the gender of the patient (Table 2).

The obtained results are shown that the *IL1B* gene variants to determine the risk of disease and predict the complication course for male patients. For female patients, it was found that in the presence of 3954TT genotype in *IL1B* gene there was no recurrence. The 108TT genotype in the *PON1* gene increased the risk of developing osteomyelitis regardless of gender.

We selected *IL1B* gene C3954T variant for this study as it is known as "the driver of inflammatory response", which expression increases activation of the cytokine cascade [26]. The modifying effect of this gene variant on the increased risk of developing bacterial traumatic osteomyelitis has been both proven [10] and refuted in some population studies [25]. But the distinct effect of this gene variant on the activity of the corresponding enzyme deserves special attention, with its increasing 4 times for carriers of the minor genotype compared to the wild type. In addition to the inflammatory reaction, it is known that the cytokine IL-1β has a significant effect on the processes of proliferation, differentiation and apoptosis at the cellular level [16]. Optimal secretion of the cytokine IL-1β is essential for the recovery of patients with both bacterial and non-bacterial osteomyelitis. Some authors have reported abnormal regulation of the inflammatory response in patients with nonbacterial osteomyelitis during active disease and remission due to increased production of IL-1β mRNA compared to controls [20]. Studying *IL1B* (C3954T) gene variants for patients with bacterial osteomyelitis we confirmed this result, finding out that among surveyed patients there is significantly predominant detection frequency of minor 3954TT genotype in the overall group of patients without complications. It is known this genotype is associated with the most active cytokine IL-1β and, correspondingly, increased production of other cytokines. In addition, we found gender differences by analyzing the distribution of *IL1B* genotypes among men and women.

In men, the frequency of 3954CC genotype distribution in *IL1B* gene was significantly increased when was complicated course. 3954CT genotype was associated in men with a reduced risk of developing both osteomyelitis and its complications. And in female patients, association of 3954TT genotype with uncomplicated course of the disease were found. So, the development of osteomyelitis and its relapsing course may be the result dysregulation or depletion of inflammatory reaction or process of apoptosis due to genetic differences between patients.

On the other hand, the genetic effect we have identified may be due to the interaction of host genes with pathogens in the infectious focus or in the circulation [7], when more intense production of proinflammatory cytokine has a protective effect against disease progression and recurrence. Considering this, it may be necessary to approach the use of monoclonal antibodies in the treatment of bacterial osteomyelitis as opposed to non-bacterial [13]. But it should be remembered that with prolonged excessive production of the cytokine IL-1β, bone resorption can be observed [13]. Therefore, patients with a better prognosis, given the variant of the *IL1B* gene, nevertheless, need monitoring and preventive measures considering these particularities.

Another aspect of our research was to study the clinical effects of *PON1* (C108T) gene variants on development and course of the disease under consideration. Since many studies have shown that when osteomyelitis occurs, systemic oxidative stress enhances as a result of an imbalance between oxidants and antioxidants in the direction of oxidized forms and peroxidation of lipids grows, for the deactivation of which paraoxanase physiological expression is extremely important [9, 11]. As a result of our study, the association of the 108TT *PON1* gene genotype with an increased risk of osteomyelitis developing was revealed. We did not find in the literature any data from similar studies and, therefore, we cannot compare the results. However, research groups have shown that patients with osteomyelitis have a decrease in serum paraoxonase activity and an increase in the concentration of lipid hydroperoxides [21]. And these data are indirectly confirmed by our results, because carriers the 108TT genotype in the *PON1* gene have a lower level of PON1 enzyme activity. Decreased enzyme activity may due to complications in patients after osteosynthesis by rising the products of oxidative stress, including the additional effects of harmful environmental factors [17].

Conclusion. We identified the association between 108TT genotype in *PON* gene with increased risk of osteomyelitis development and the association of 3954TT genotype in *IL1B* gene with a decreased risk of recurrent course of osteomyelitis. Gender differences were found in the clinical effects of *IL1B* gene variants: in men, the prevalence of 3954CC genotype was significantly to be increased in recurrent course of osteomyelitis; 3954CT genotype was associated with a reduced risk of osteomyelitis and its complications developing, while in women the association of 3954TT with an uncomplicated course of the disease was found. The obtained results are promising for predicting the risk of bacterial osteomyelitis and its complications including future personalized prevention strategy development.

REFERENCES

1.1000 Genomes Project Consortium. A global reference for human genetic variation. // Nature. 2015; 526(7571): 68-74
2. Alcântara JE Junior, Aguiar RA, Sampaio JGL NETO, Azi

ML, Sadigursky D, Alencar DF. Factors associated with the development of early infection after surgical treatment of fractures. // Acta Ortop Bras. 2018; 26(1): 22-6.
3. Alves De Souza C, Queiroz Alves De Souza A, Queiroz Alves De Souza MDS, Dias Leite JA, Silva De Morais M, Barem Rabenhorst SH. A link between osteomyelitis and IL1RN and IL1B polymorphisms-a study in patients from Northeast Brazil. // Acta Orthop. 2017; 88 (5): 556-61.
4. Carter KW, Hung J, Powell BL, Wiltshire S, Foo BT, Leow YC, et al. Association of Interleukin-1 gene polymorphisms with central obesity and metabolic syndrome in a coronary heart disease population. // Hum Genet. 2008; 124 (3): 199-206.
5. Chin KY, Wong SK, Ekeuku SO, Pang KL. Relationship Between Metabolic Syndrome and Bone Health –An Evaluation of Epidemiological Studies and Mechanisms Involved. // Diabetes Metab Syndrome Obes. 2020; 13: 3667-90.
6. Cox AJ, Ferguson PJ. Update on the genetics of nonbacterial osteomyelitis in humans.// Curr Opin Rheumatol. 2018; 30 (5): 521-5.
7. Ferreira GF, Moraes C, Silveira AM, Correa-Oliveira R, Teixeira-Carvalho A, Martins-Filho OA, et al. Distinct cytokine profiles of circulating mononuclear cells stimulated with Staphylococcus aureus enterotoxin A in vitro during early and late episodes of chronic osteomyelitis. // Mem Inst Oswaldo Cruz. 2012; 107 (3): 348-55.
8. Fu P, Xie S, Zhang X. Investigation of the IL-1 β +3954 C> T polymorphism and the risk of sepsis. // Medicine. 2020; 99 (31): e21022.
9. Grbic R, Miric DJ, Kisic B, Popovic L, Nestorovic V, Vasic A. Sequential analysis of oxidative stress markers and vitamin C status in acute bacterial osteomyelitis. // Mediators Inflamm. 2014; 2014: 975061.
10. Jiang N, Li SY, Ma YF, Hu YJ, Lin QR, Yu B. Associations between Interleukin Gene Polymorphisms and Risks of Developing Extremity Posttraumatic Osteomyelitis in Chinese Han Population. // Mediators Inflamm. 2020; 5: 3278081.
11. Jyoti A, Singh S, Mukhopadhyay B, Gavel R, Mishra SP. Free radicals and antioxidant status in chronic osteomyelitis patients: a case control study. // J Clin Diagn Res. 2015; 9 (4): BC 08-10.
12. Kremers HM, Nwojo ME, Ransom JE, Wood-Wentz CM, Melton LJ 3rd, Huddleston PM 3rd. Trends in the epidemiology of osteomyelitis: a population-based study, 1969 to 2009. // J Bone Joint Surg Am. 2015; 97 (10): 837-45.
13. Longo B, Tonial AF, Skare T. Use of infliximab in patient with chronic non - bacterial osteomyelitis, case report.// Archivos de Medicina. 2019, 19 (1): 168-72.
14. Mackness B, Durrington PN, Mackness MI. Human paraoxonase serum. // Gene Pharmacol. 1998; 31 (3): 329-36.
15. Oliveira PR, Carvalho VC, da Silva Felix C, de Paula AP, Santos-Silva J, Lima AL. The incidence and microbiological profile of surgical site infections following internal fixation of closed and open fractures. // Re in Bras Ortop. 2016; 51 (4): 396-9.
16. Open database: Gene. Integrates information from a wide range of species [Internet]. Bethesda (MD): National Center for Biotechnology Information (US); c2005- [cited 20 21 June 15]. Available from: https://www.ncbi.nlm.nih.gov/gene/3553
17. Piras IS, Gabriele S, Altieri L, Lombardi F, Sacco R, Lintas C, et al. Reevaluation of Serum Arylesterase Activity in Neurodevelopmental Disorders. // Antioxidants (Basel). 2021; 10 (2): 164.
18. Richards JE, Kauffmann RM, Zuckerman SL, Obremskey WT, May AK. Relationship of hyperglycemia and surgical-site infection in orthopedic surgery. //J Bone Joint Surg Am. 2012; 94 (13): 1181-6.
19. Saeidi M, Shakeri R, Marjani A, Khajeniazi S. Alzheimer's Disease and Paraoxonase 1 (PON1) Gene Polymorphisms. // Open Biochem J. 2017; 11: 47-55.
20. Scianaro R, Insalaco A, Bracci Laudiero L, De Vito R, Pezzullo M, Teti A, et al. Deregulation of the IL-1 β axis in chronic recurrent multifocal osteomyelitis. // Pediatr Rheumatol Online J. 2014; 12:30.
21. Tekin Koruk S, Aksoy N, Hamidanoglu M, Karsen H, Unlu S, Bilinc H. The activity of paraoxonase and arylesterase in patients with osteomyelitis. // Scand J Clin Lab Invest. 2012; 72 (7): 513-7.
22. Trampuz A, Zimmerli W. Diagnosis and treatment of infections associated with fracture-fixation devices. // Injury. 2006; 37 (2): S59 - 66.
23. Tribble DR, Lewandowski LR, Potter BK, Petfield JL, Stinner DJ, Ganesan A, et al. Trauma infectious disease outcomes study group. osteomyelitis risk factors related to combat trauma open tibia fractures: a case-control analysis.// J Orthop Trauma. 2018; 32 (9): e 344-53.
24. Valle-Garay E, Montes AH, Corte JR, Meana A, Fierer J, Asensi V. tPA Alu (I / D) polymorphism associates with bacterial osteomyelitis. // J Infect Dis. 2013; 208 (2): 218-23.
25. Yao ZL, Lin QR, Hu YJ, Hou YL, Ma YF, Yu B, et al. Interleukin-1 Beta Gene Polymorphism rs16944 May Associate with Increased Susceptibility to Extremity Chronic Osteomyelitis in Chinese Han Population. // Biomed Res Int. 2019; 2019: 7483537.
26. Young S, Sharma N, Lee JH, Chitu V, Neumeister V, Sohr E, et al. Mast cells enhance sterile inflammation in chronic nonbacterial osteomyelitis. // Dis Model Mech. 2019; 12 (8): dmm 040097.

SUMMARY

VARIANTS OF *IL1* (C3954T, RS1143634), *PON1* (C108T, RS705379) GENES AS PROGNOSTIC MARKERS OF OSTEOMYELITIS RISK AND ITS COMPLICATIONS

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The aim of the work was to study the effect of *IL1B* (C3954T, rs1143634), *PON1* (C108T, rs705379) gene variants on the risk of bacterial osteomyelitis development and its complicated course. The study involved 56 patients with osteomyelitis – 20 with not complicated (non-recurrent) course after treatment and 36 with complicated (recurrent) course. The data of population frequencies for the European population, obtained from the open database of "1000 Genomes project", were used as a control group.

There was significantly increased distribution frequency of genotype 3954TT of *IL1B* in patients with uncomplicated course compared to the control group (χ^2=6.05, p=0.014, OR=4.99 (1.55-16.07)). And was found increased of minor genotype 108TT of *PON1* frequency in patients with osteomyelitis compared to control group (χ^2=4.38, p=0.036, OR=1.85(1.03-3.33)).

There were found gender differences in the clinical effects of

IL1B gene variant: in men, the prevalence of genotype 3954CC was significantly to be increased in the patient with complicated osteomyelitis; genotype 3954CT was associated with a reduced risk of osteomyelitis and its complications developing, while in women was found the association of genotype 3954TT with an uncomplicated course of the disease.

In conclusion, this study suggests that the variants of *IL1B* and *PON1* genes associated with the risk of developing bacterial osteomyelitis and its complicated course and can be used as a prognostic marker for developing personalized prevention strategies.

Keywords: osteomyelitis, *IL1B*, *PON1*, gene, gender.

РЕЗЮМЕ

ВАРИАНТЫ ГЕНОВ *IL1B (C3954T,* RS1143634), *PON1* (C108T, RS705379) КАК ПРОГНОСТИЧЕСКИЕ МАРКЕРЫ РИСКА ОСТЕОМИЕЛИТА И ЕГО ОСЛОЖНЕНИЙ

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Цель исследования – изучить влияние вариантов генов *IL1*B (C3954T, rs1143634), *PON1* (C108T, rs705379) на риск развития бактериального остеомиелита и его осложнений. Обследовано 56 пациентов с остеомиелитом: 20 с неосложненным течением после лечения и 36 - с осложненным (рецидивирующим) течением. В качестве контрольной группы использованы данные о частотах популяций для европейского населения, полученные из открытой базы данных «1000 Genomes project».

Средний возраст пациентов составил 43,1±14,6 г., из них 39 (69,6%) мужчин и 17 (30,4%) женщин. Выявлено достоверное увеличение частоты распределения генотипа 3954TT гена *IL1*B у пациентов с неосложненным течением в сравнении с контрольной группой (χ^2=6,05, p=0,014, OR=4,99 (1,55-16,07)), а также повышение частоты минорного генотипа 108TT гена *PON1* у пациентов с остеомиелитом в сравнении с контрольной группой (χ^2=4,38, p=0,036, OR=1,85 (1,03–3,33)).

Обнаружены гендерные различия в клинических эффектах варианта гена *IL1*B: у мужчин распространенность генотипа 3954CC достоверно повышена при наличии осложненного остеомиелита; генотип 3954CT ассоциирован со снижением риска развития остеомиелита и его осложнений, тогда как у женщин обнаружена ассоциация генотипа 3954TT с неосложненным течением заболевания.

Результаты проведенного исследования позволяют заключить, что варианты генов *IL1B* и *PON1* ассоциированы с риском развития бактериального остеомиелита, его осложнённого течения, что может быть использовано в качестве прогностического маркера при разработке персонализированных стратегий профилактики.

რეზიუმე

გენების *IL1B* (C3954T, RS1143634), *PON1* (C108T, RS705379) ვარიანტები, როგორც ოსტეომიელიტის რისკის და მისი გართულებების პროგნოზული მარკერები

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კვლევის მიზანს წარმოადგენდა გენების *IL1B* (C3954T, RS1143634), *PON1* (C108T, RS705379) ვარიანტების გავლენის შეფასება ბაქტერიული ოსტეომიელიტის და მისი გართულებების განვითარების რისკზე. გამოკვლეულია 56 პაციენტი ოსტეომიელიტით: 20 - გაურთულებელი მიმდინარეობით მკურნალობის შემდეგ, 36 - გართულებული (მორეციდივე) მიმდინარეობით. საკონტროლო ჯგუფად გამოყენებული იყო მონაცემები პოპულაციური სიხშირის შესახებ ევროპის მოსახლეობისათვის, მიღებული მონაცემთა ღია ბაზიდან "1000 Genomes project".

პაციენტების საშუალო ასაკმა შეადგინა 43,1±14,6 წ., მათგან 39 (69,9%) მამაკაცი, 17 (30,4%) ქალი. გამოვლენილია გენი *IL1B*-ის 3954TT გენოტიპის განაწილების სიხშირის მატება პაციენტებში გაურთულებული მიმდინარეობით, საკონტროლო ჯგუფთან შედარებით (χ^2=6,05, p=0,014, OR=4,99 (1,55-16,07)), ასევე, გენი *PON1*-ის მინორული გენოტიპის 108TT სიხშირის ზრდა პაციენტებში ოსტეომიელიტით საკონტროლო ჯგუფთან შედარებით (χ^2=4,38, p=0,036, OR=1,85 (1,03–3,33)).

აღმოჩენილია გენდერული განსხვავებანი გენი *IL1B*-ის ვარიანტების კლინიკურ ეფექტებში: მამაკაცებში გენოტიპი 3954CC-ის გავრცელება სარწმუნოდ მომატებულია გართულებული ოსტეომიელიტის არსებობის პირობებში; გენოტიპი 3954CT ასოცირებულია ოსტეომიელიტის და მისი გართულებების განვითარების რისკის შემცირებასთან, ხოლო ქალებში დადგენილია გენოტიპი 3954TT-ის ასოციაცია დაავადების გაურთულებელ მიმდინარეობასთან.

ჩატარებული კვლევის შედეგები იძლევა საფუძველს დასკვნისათვის, რომ გენების *IL1*B და *PON1* ვარიანტები ასოცირებულია ბაქტერიული ოსტეომიელიტის განვითარების, მისი გართულებული მიმდინარეობის რისკთან, რაც შეიძლება გამოყენებული იქნას პროგნოზულ მარკერად პროფილაქტიკის პერსონალიზებული სტრატეგიების შემუშავების დროს.