DOI: 10.20514/2226-6704-2024-14-1-63-71
УДК 578.834.1
EDN: OPXUDO

А.М. Караченова*, Е.Н. Романова

Федеральное государственное бюджетное образовательное учреждение высшего образования «Читинская государственная медицинская академия» Министерства здравоохранения Российской Федерации, кафедра поликлинической терапии с курсом медицинской реабилитации, Чита, Россия

ВЛИЯНИЕ УРОВНЯ ВИТАМИНА D И ПОЛИМОРФИЗМА ГЕНА ЕГО РЕЦЕПТОРА (BSMI, FOKI) НА ТЯЖЕСТЬ COVID-19-АССОЦИИРОВАННОГО ПОРАЖЕНИЯ ЛЕГКИХ

A.M. Karachenova*, E.N. Romanova

Chita State Medical Academy, Department of polyclinic therapy with a course of medical rehabilitation, Chita, Russia

Effect of Vitamin D Receptor Gene Polymorphism (BsmI, FokI) and its Concentration on the Severity of Covid-Associated Lung Damage

Резюме

Цель. Выявить взаимосвязь между сывороточным содержанием витамина D и полиморфизмом гена рецептора витамина D с тяжестью течения COVID-19-ассоциированного поражения легких. **Материалы и методы.** В работе представлены результаты обследования 200 человек через 1 месяц после перенесенного COVID-ассоциированного поражения легких в период с 01 июня по 31 октября 2020 года. Пациенты разделены на группы по 50 человек в зависимости от степени поражения легких по результатам проведения компьютерной томографии: 1-я группа (КТ-1), медиана по возрасту составила 51,5 [50,5; 54,8]; 2-я группа (КТ-2), медиана по возрасту 57,0 [53,1; 57,0]; 3-я группа (КТ-3), медиана по возрасту 52,5 [51,9; 55,0]; 4-я группа (КТ-4), медиана 55,0 [53,2; 56,4]. В группу контроля вошли 56 человек относительно здоровых лиц, не болевших коронавирусной инфекцией, медиана по возрасту составила 55,0 [51,1; 55,0]. Все группы были сопоставимы по возрасту и полу. В сыворотке крови исследовали концентрацию общего 25-гидроксивитамина D (25(OH)D). Также проведено молекулярно-генетическое исследование гена рецептора витамина D: 283 A>G (BsmI) и 2 A>G (FokI). **Результаты.** Учитывая полученные результаты у пациентов, перенесших COVID-19-ассоциированное поражение легких, можно предположить, что недостаточное содержание в крови общего 25-гидроксивитамина D может являться одним из факторов, способствующих осложненному течению коронавирусной инфекции, а также фактором риска ухудшения течения COVID-19-ассоциированного поражения легких. Анализ полиморфизма гена рецептора витамина D VDR: 283 A>G показал преимущественное наследование аллели A и гомозиготы A/A у пациентов с большим уровнем повреждения легочной ткани на фоне COVID-19 инфекции — КТ-3, 4. Изучение полиморфизма гена рецептора витамина D VDR: 2 A>G показало преимущественное наследование гомозиготы A/A среди заболевших по сравнению с группой контроля. При изучении концентрации витамина D у пациентов с COVID-19-ассоциированным поражением легких в зависимости от полиморфизма генов рецептора витамин D VDR: 283 A>G (BsmI) и VDR: 2 A>G (FokI) отличий не выявлено. **Заключение.** Недостаточное содержание в крови 25(OH)D может являться одним из факторов, способствующих осложненному течению коронавирусной инфекции. Анализ полиморфизма гена рецептора витамина D VDR: 283 A>G показал преимущественное наследование аллели A и гомозиготы A/A у более тяжелой категории пациентов — с объемом повреждения легочной ткани более 50 % (КТ-3, 4) на фоне COVID-19 инфекции. Изучение полиморфизма гена рецептора витамина D VDR: 2 A>G выявило среди заболевших наиболее распространенное носительство гомозиготы A/A по сравнению с группой контроля.

Ключевые слова: *COVID-19-ассоциированное поражение легких, полиморфизм гена рецептора витамина D: 283 A>G (BsmI) и 2 A>G (FokI)*

Конфликт интересов

Авторы заявляют, что данная работа, её тема, предмет и содержание не затрагивают конкурирующих интересов

*Контакты: Анастасия Михайловна Караченова, e-mail: b_a_m_2010@mail.ru
*Contacts: Anastasia M. Karachenova, e-mail: b_a_m_2010@mail.ru

Источники финансирования

Работа выполнена при финансовой поддержке ФГБОУ ВО Читинская государственная медицинская академия Минздрава РФ в рамках утвержденного плана НИР

Статья получена 26.12.2023 г.

Принята к публикации 26.01.2024 г.

Для цитирования: Караченова А.М., Романова Е.Н. ВЛИЯНИЕ УРОВНЯ ВИТАМИНА D И ПОЛИМОРФИЗМА ГЕНА ЕГО РЕЦЕПТОРА (BsmI, FokI) НА ТЯЖЕСТЬ COVID-19-АССОЦИИРОВАННОГО ПОРАЖЕНИЯ ЛЕГКИХ. Архивъ внутренней медицины. 2024; 14(1): 63-71. DOI: 10.20514/2226-6704-2024-14-1-63-71. EDN: OPXUDO

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Objective. To identify the relationship between the serum vitamin B content and the polymorphism of the vitamin B receptor gene with the severity of the course of COVID-19-associated lung damage.

To identify the relationship between serum vitamin D content and polymorphism of the vitamin D receptor gene with the severity of COVID-19-associated lung damage. **Materials and methods.** The paper presents the results of an examination of 200 people, after 1 month suffering COVID-associated lung damage in the period from June 1 to October 31, 2020. The patients were divided into groups of 50 people depending on the degree of lung damage based on the results of computed tomography: group 1 (CT-1), median by age was 51.5 [50.5; 54.8]; group 2 (CT-2), median by age 57.0 [53.1; 57.0]; group 3 (CT-3), median by age 52.5 [51.9; 55.0]; group 4 (CT-4), median 55.0 [53.2; 56.4]. The control group included 56 relatively healthy people who did not have coronavirus infection; the median age was 55.0 [51.1; 55.0]. All groups were comparable in age and gender. The concentration of total 25-hydroxyvitamin D (25(OH)D) was studied in blood serum. A molecular genetic study of the vitamin D receptor gene was also carried out: 283 A>G (BsmI) and 2 A>G (FokI). **Results.** It was revealed that insufficient levels of 25(OH)D in the blood are one of the risk factors for the development of COVID-19 infection, as well as a risk factor for worsening the course of COVID-19-associated lung damage. Analysis of the polymorphism of the vitamin D receptor gene VDR: 283 A>G showed the predominant inheritance of allele A and homozygote A/A in patients with a high level of damage to lung tissue due to COVID-19 infection — KT-3, 4. Study of polymorphism of the vitamin D receptor gene VDR: 2 A>G showed preferential inheritance of homozygote A/A among patients compared to the control group. When studying the concentration of vitamin D in patients with COVID-19-associated lung damage depending on the polymorphism of the vitamin D receptor genes VDR: 283 A>G (BsmI) and VDR: 2 A>G (FokI), no differences were found. **Conclusion.** Insufficient levels of 25(OH)D in the blood may be one of the factors contributing to the complicated course of coronavirus infection. Analysis of the vitamin D receptor gene polymorphism VDR: 283 A>G showed preferential inheritance of the A allele and homozygote A/A in a more severe category of patients — with more than 50 % damage to the lung tissue (CT-3, 4) against the background of COVID-19 infection. A study of the polymorphism of the vitamin D receptor gene VDR: 2 A>G revealed the most common carriage of the A/A homozygote among patients compared to the control group.

Key words: *COVID-19-associated lung damage, vitamin D receptor gene polymorphism: 283 A>G (BsmI) and 2 A>G (FokI)*

Conflict of interests

The authors declare no conflict of interests

Sources of funding

The work was carried out with the financial support of the Chita State Medical Academy of the Ministry of Health of the Russian Federation within the framework of the approved research plan

Article received on 26.12.2023

Accepted for publication on 26.01.2024

For citation: Karachenova A.M., Romanova E.N. Effect of Vitamin D Receptor Gene Polymorphism (BsmI, FokI) and its Concentration on the Severity of Covid-Associated Lung Damage. The Russian Archives of Internal Medicine. 2024; 14(1): 63-71. DOI: 10.20514/2226-6704-2024-14-1-63-71. EDN: OPXUDO

Introduction

Vitamin D is one of the key immunity regulators [1, 2]. Vitamin D deficiency observed in 80 % of the Russian citizens is associated with impaired innate and acquired immunity, resulting in a high risk of viral and bacterial infections. Patients with vitamin D deficiency have significantly reduced resistance to bacterial and viral diseases (ARVI, flu, rhinitis, obstructive pulmonary diseases) [2, 3].

Recent studies conducted during and after the COVID-19 pandemic demonstrated that vitamin D deficiency can contribute to the morbidity rates and aggravate SARS-CoV-2 infection [4-6]. It has been shown that there are several mechanisms of how vitamin D can reduce the risk of bacterial and viral infections by creating a barrier and affecting the innate cellular and humoral immunity [7]. An active form of vitamin D (calcitriol 1.25(OH)2D3) facilitates the reduction in pro-inflammatory cytokine (IL-6, TNFα, CXCL8, CXCL10) levels, stimulates synthesis of antimicrobial peptides (cathelicidin, defensin), which have antiviral effects [3, 8]. Also, one of the vitamin D functions is related to pathogen identification by macrophages, it being innate immune response. Besides, vitamin D suppresses IL-2 and IFγ production by type 1 T-helper cells and stimulates cytokine production by type 2 T-helper cells [9]. Given that vitamin D takes part in the activity of the renin-angiotensin-aldosterone system, it is assumed that it controls the amount of mRNA (messenger ribonucleic

acid) and expression of angiotensin-converting enzyme-2, responsible for the protective against various respiratory infections [10]. As for SARS-CoV-2, it is worth mentioning that vitamin D can suppress supposed adhesive molecules (DPP-4/CD26) for viral penetration to the cell [6, 10]. Vitamin D affects the body with the help of the VDR (vitamin D receptor), localised on chromosome 12, locus 12q13.11 [11]. VDR is specifically activated by calcitriol and causes changes in expression of over 2,700 human genes [2, 3]. A genome-wide biological system analysis by Gromova OA et al. to study the VDR binding made it possible to systematise biological roles of vitamin D for further treatment and prevention of a wide array of diseases [12]. It has been found out that the antiviral immunity is supported by at least 155 proteins, the expression of which is regulated by vitamin D receptor [3, 12].

Vitamin D receptor is characterised by polymorphism, i.e. various allelic variants of this gene in a population [13, 14]. *VDR* gene localised on chromosome 12q13.11 contains a number of single nucleotide polymorphisms, including polymorphism 283 A>G (BsmI) (rs1544410), polymorphism 2 A>G (FokI) (rs2228570). The Russian scientific literature does not contain any data on the study of the correlation between respiratory pathologies and polymorphisms BsmI and FokI of the *VDR* gene; however, there are foreign studies discussing this correlation with bronchopulmonary diseases [11].

Study Objective

To find the correlation between serum vitamin D levels and polymorphism of the vitamin D receptor gene and the severity of COVID-19-associated lung involvement.

Materials and Methods

The study included 200 patients with a history of COVID-19-associated lung involvement during the period from June 1 to October 31, 2020 one month after discharge from in-patient clinics in Chita. All patients were divided into groups of 50 people, depending on the degree of pulmonary involvement as seen on CT scans: group 1 (CT1) — median age was 51.5 [50.5; 54.8]; group 2 (CT2) — median age was 57.0 [53.1; 57.0]; group 3 (CT3) — median age was 52.5 [51.9; 55.0]; group 4 (CT4) — median age was 55.0 [53.2; 56.4]. The study enrolled patients with confirmed novel coronavirus infection, where SARS-CoV-2 (Severe Acute Respiratory Syndrome-relate Coronavirus 2) RNA was identified with real-time polymerase chain reaction. Exclusion criteria were: lymph and myeloproliferative disorders, system diseases requiring immunosuppression therapy, HIV infection, chronic alcoholism, pregnancy, intake of vitamin D.

The control group included 56 healthy volunteers without a history of coronavirus infection and other respiratory diseases within the past three months; median age was 55.0 [51.1; 55.0]. All study groups were similar in sex and age composition.

Serum vitamin D (total 25-hydroxyvitamin D) was measured by an immunoassay after collection of serum samples from all study subjects. Serum 25(OH)D levels were measured using commercially available kits and Access 2 analyser (Beckmancoulter, USA). Molecular genetic testing of vitamin D receptor gene 283 A>G (BsmI) (rs1544410) and 2 A>G (FokI) (rs 2228570) was performed using polymerase chain reaction with allele-specific primer (LEGEND plex™). DNA was isolated with real-time PCR (PCR-RT) and PCR with electrophoretic result detection (DNA Technology).

The study was approved by the Ethics Committee at the Federal State Budgetary Educational Institution of Higher Education Chita State Medical Academy of the Ministry of Health of the Russian Federation. Before any assessments, patients provided their voluntary informed consent; all activities were performed in accordance with the World Health Organisation's Declaration of Helsinki (2013).

Statistical processing of study results was performed with IBM SPSS Statistics Version 25.0 (licence No. Z125-3301-14, IBM, USA). Statistical analysis followed the principles of the International Committee of Medical Journal Editors (IC MJE) and the Statistical Analysis and Methods in the Published Literature (SAMPL) guidelines. Normality of parameter distribution in groups of over 50 subjects was assessed using the Kolmogorov–Smirnov test. Given that the parameter distribution in all study groups was not normal, obtained data were presented as a median value, first and third quartiles: Me [Q_1; Q_3]. Kruskal-Wallis test was used to compare quantitative parameters in three independent groups. Results were statistically significant at $p < 0.05$. Pair-wise comparison of two independent groups using one quantitative parameter, Bonferroni modified Mann–Whitney U test was used. Correlation relationships between study parameters were identified with the help of the Spearman's coefficient. The degree of correlation between study parameters was found using Chaddock scale. Ratings were described with absolute values and percentages. Study ratings were compared with the use of Pearson's χ^2 which allows assessing the significance of differences between the actual number of outcomes or qualitative characteristics of a group in each category and the theoretical number which can be expected in the study groups if the zero hypothesis is true. For smaller groups, likelihood-adjusted Pearson's chi-squared test was preferable. Cramer's factor (V) was used to measure the degree of correlation between the risk factor and outcome. The distribution of the frequency of vitamin D genotypes 283 A>G (BsmI) and 2 A>G (FokI) corresponded to Hardy-Weinberg equilibrium [14].

Results and Discussion

Analysis of vitamin D concentration in the study groups demonstrated lower levels in patients with COVID-19-associated lung involvement vs. controls. Difference vs. group 1 was 1.2-fold [1.14; 1.22] ($p < 0.001$), group 2 — 1.3-fold [1.22; 1.31] ($p < 0.001$), group 3 — 1.4 fold [1.29; 1.38] ($p < 0.001$), and group 4 — 1.4 -fold [1.34; 1.45] ($p < 0.001$) (Table 1). Also, lower levels of vitamin D were found in patients with an extensive pulmonary tissue involvement: in groups 3 (CT-3) and 4 (CT-4) vs. group 1 (CT-1) — 1.12-fold [1.09; 1.17] and 1.17-fold [1.13; 1.23], respectively ($p < 0.001$); in group 4 (CT-4) vs. group 2 (CT-2) — 1.12-fold [1.06; 1.15] ($p < 0.001$) (Table 1).

According to the 2021 Vitamin D Deficiency Guidelines [16] in adults, measurement of vitamin D levels in patients corresponded to the following criteria: vitamin D deficiency — blood 25(OH)D level of < 20 ng/mL, inadequate vitamin D level — blood 25(OH)D level between ≥ 20 and < 30 ng/mL, with the target value of 30–60 ng/mL. In our study, low vitamin D levels were recorded in 184 patients (92 %): group 1 (CT-1) — 41 (82 %) subjects, group 2 (CT-2) — 46 (92 %) subjects, group 3 (CT-3) — 48 (96 %) subjects, group 4 (CT-4) — 49 (98 %) subjects. Besides, vitamin D deficiency was diagnosed in 2 patients in group CT-3 (4 %) and 7 patients (14 %) in group CT-4. The majority of controls had target 25(OH)D levels (87.5 %), unlike patients who had coronavirus infection. This value was 4.9 times higher than in group 1 ($p < 0.001$), 10.9 times higher than in group 2 ($p < 0.001$), 21.9 times higher than in group 3 ($p < 0.001$), 43.8 times higher than in group 4 ($p < 0.001$) (Table 2).

In analysis of the group of patients, depending on the severity of COVID-19-associated lung involvement, target vitamin D levels in group 1 (CT-1) vs. group 3 (CT-3) and 4 (CT-4) were found 4.5 times ($p = 0.03$) and 9 times ($p = 0.02$) more often (Table 2).

The correlation analysis demonstrated that there is moderate inverse relationship ($V = -0.46$, $p < 0.001$) between 25(OH)D level and extent of lung tissue involvement.

Therefore, it can be assumed that lower serum vitamin D levels are a risk factor of coronavirus infection and a risk factor of aggravated COVID-19-associated lung involvement.

Table 1. *The concentration of vitamin D in the blood of patients of the studied groups*

Groups		Vitamin D concentration, ng/ml Me [Q1; Q3]	Statistics		
			Kruskal-Wallis	Manna-Whitney	
				Comparison with control group	Comparison of groups studied
Control group, n=56	к	33,17 [32,46; 33,53]	H=130,53, df=4, P <0,001.	**$U_{к-1}$=397,5, $p_{к-1}$<0,001; $U_{к-2}$=172,0, $p_{к-2}$<0,001; $U_{к-3}$=96,5, $p_{к-3}$<0,001; $U_{к-4}$=73,5, $p_{к-4}$<0,001.**	**U_{1-2}=907,0, p_{1-2}=0,02; U_{1-3} =512,0, p_{1-3}<0,001; U_{1-4} =421,0, p_{1-4}<0,001; U_{2-3} =861,0, p_{2-3}=0,007; U_{2-4} =702,0, p_{2-4}<0,001;** U_{3-4} =1010,0, p_{3-4}=0,1.
Group 1, n=50	1	27,53 [27,41; 28,43]			
Group 2, n=50	2	26,41 [25,65; 26,61]			
Group 3, n=50	3	24,54 [24,23; 25,11]			
Group 4, n=50	4	23,51 [23,17; 24,19]			

Note: the statistical significance of the differences between: $p_{к-1}$ — control group and group 1; $p_{к-2}$ — control group and group 2; $p_{к-3}$ — control group and group 3; $p_{к-4}$ — control group and group 4; p_{1-2} — between 1 and 2 groups of patients; p_{1-3} — between 1 and 3 groups of patients; p_{1-4} — between 1 and 4 groups of patients; p_{2-3} — between 2 and 3 groups of patients; p_{2-4} — between 2 and 4 groups of patients; p_{3-4} — between 3 and 4 groups of patients

Table 2. *Characteristics of patients depending on the level of vitamin D concentration*

Groups		Number of patients with low 25(OH)D levels (less than 30 ng/ml)	Number of patients with target level 25(OH)D (from 30 to 60 ng/ml)	Statistics P χ^2
Control group, n=56	к	12,5 % (7/56)	87,5 % (49/56)	**$\chi^2_{к-1}$=51,5; $p_{к-1}$<0,001; $\chi^2_{к-2}$=66,8; $p_{к-2}$<0,001;**
Group 1, n=50	1	82 % (41/50)	18 % (9/50)	**$\chi^2_{к-3}$=24,1; $p_{к3}$<0,001; $\chi^2_{к-4}$=23,58; $p_{к-4}$<0,001.**
Group 2, n=50	2	92 % (46/50)	8 % (4/50)	χ^2_{1-2}=1,4; p_{1-2}= 0,14; **χ^2_{1-3}=5,01; p_{1-3}=0,03;**
Group 3, n=50	3	96 % (48/50)	4 % (2/50)	**χ^2_{1-4}=5,4; p_{1-4}=0,02.** F_{2-3}=0,7; p_{2-3}=0,68
Group 4, n=50	4	98 % (49/50)	2 % (1/50)	F_{2-4}=1,47; p_{2-4}=0,21 F_{3-4}=0,6p_{3-4}=0,62

Note: see table 1

Scientific literature contains similar information that higher serum vitamin D concentrations are associated with a reduced risk and milder COVID-19 infection [17]. Also, there is evidence that vitamin D activates immune cells, which are then used to produce immune peptides and proteins — cathelicidins and defensins, which have an array of antimicrobial and antiviral effects [18, 19].

In turn, it would be interesting to study the association between the levels of vitamin D with known polymorphisms of vitamin D gene depending on the severity of COVID-19-associated lung involvement. We managed to perform a genetic testing of 156 patients; thus, groups 1, 2 (CT-1,2) — group I were compared with groups 3, 4 (CT-3,4) — group II.

Analysis of polymorphism of vitamin D receptor gene VDR: 283 A>G in patients with COVID-19-associated lung involvement demonstrated that allele G is 1.2 times more common in patients with less extended pulmonary tissue involvement (CT-1,2) vs. controls ($p < 0.03$; OR = 0.6). Also, it was found out that patients with less extended pulmonary tissue involvement (CT-1,2) have allele G 1.4 times more often ($p < 0.001$; OR = 2.5) than controls (CT-3,4). Patients with more extended lung tissue involvement (CT-3,4) have allele A 1.8 times more often ($p < 0.001$; OR = 0.4). Analysis of polymorphism genotypes of vitamin D receptor gene VDR: 283 A>G demonstrated that polymorphism G/G is 1.7 times more common in groups CT-1,2 vs. controls ($p = 0.01$; OR = 0.4). The study of polymorphism A/G showed that it is 1.6 times more common in controls vs. groups CT-1,2 ($p = 0.02$; OR = 2.3) and 1.3 times more common vs. groups CT-3,4 ($p = 0.12$; OR = 1.7). Polymorphism A/A is observed mostly in more severe cases of COVID-19-associated lung involvement (group II) (2.8 times more common) ($p = 0.006$; OR = 0.3) vs. patients with less extended lung involvement (group I) (Table 3).

The study of polymorphism of vitamin D receptor gene VDR: 2 A>G in patients with lung involvement associated with past COVID-19 infection demonstrated predominant inheritance of homozygote A/A in groups I and II: 2.6 times more common ($p = 0.04$; OR = 0.3) and 2.5 times more common ($p = 0.04$; OR = 0.4) vs. controls, respectively. Analysis of genotype A/G of the studied polymorphism demonstrated its predominance in the control group: 1.7 times more common than in group I ($p = 0.007$; OR = 2.7) and 1.6 times more common than in group II ($p = 0.009$; OR = 2.5) (Table 3).

Table 3. *Distribution of the frequency of alleles and genotypes of the vitamin D receptor gene polymorphism VDR:283 A>G (BsmI), VDR:2 A>G (FokI) in patients with COVID-19-associated lung damage*

Gene	Genotypes and alleles	Control group n=56	Group		Statistics	Pairwise comparison of study groups
			I (KT-1, 2) n=74	II (KT-3,4) n=82		
VDR: 283 A>G	G	60,7 % (68/112)	73,6 % (109/148)	53 % (87/164)	**χ^2=14,21 df=2 p<0,001**	**$\chi^2_{к-1}$=4,91; $p_{к-1}$=0,03;** $\chi^2_{к-2}$=1,59; $p_{к-2}$=0,21; **χ^2_{1-2}=14,13; p_{1-2}<0,001;**
	A	39,3 % (44/112)	26,4 % (39/148)	47 % (77/164)		
	G/G	33,9 % (19/56)	56,8 % (42/74)	32,9 % (27/82)		**$\chi^2_{к-1}$=6,67; $p_{к-1}$=0,01;** $\chi^2_{к-2}$=0,02; $p_{к-2}$=0,90; **χ^2_{1-2}=8,96; p_{1-2}=0,003;**
	A/G	53,6 % (30/56)	33,8 % (25/74)	40,2 % (33/82)	**χ^2=17,24 df=4 p=0,002**	**$\chi^2_{к-1}$=5,11; $p_{к-1}$=0,02;** $\chi^2_{к-2}$=2,38; $p_{к-2}$=0,12; χ^2_{1-2}=0,69; p_{1-2}=0,41;
	A/A	12,5 % (7/56)	9,5 % (7/74)	26,8 % (22/82)		$\chi^2_{к-1}$=0,07; $p_{к-1}$=0,79; **$\chi^2_{к-2}$=4,12; $p_{к-2}$=0,04; χ^2_{1-2}=7,78; p_{1-2}=0,006;**
VDR:2 A>G	A	39,3 % (44/112)	41,2 % (61/148)	40,9 % (67/164)	χ^2=0,11 df=2 p=0,95	$\chi^2_{к-1}$=0,09; $p_{к-1}$=0,75; $\chi^2_{к-2}$=0,07; $p_{к-2}$=0,79; χ^2_{1-2}=0,004; p_{1-2}=0,95;
	G	60,7 % (68/112)	58,8 % (87/148)	59,1 % (97/164)		
	A/A	8,9 % (5/56)	23,0 % (17/74)	22 % (18/82)		**$\chi^2_{к-1}$=3,53; $p_{к-1}$=0,04; $\chi^2_{к-2}$=3,18; $p_{к-2}$=0,04;** χ^2_{1-2}=0,02; p_{1-2}=0,88;
	A/G	60,7 % (34/56)	36,5 % (27/74)	37,8 % (31/82)	**χ^2=10,38 df=4 p=0,035**	**$\chi^2_{к-1}$=7,51; $p_{к-1}$=0,007; $\chi^2_{к-2}$=7,01; $p_{к-2}$=0,009;** χ^2_{1-2}=0,03; p_{1-2}=0,87;
	G/G	30,4 % (17/56)	40,5 % (30/74)	40,2 % (33/82)		$\chi^2_{к-1}$=1,43; $p_{к-1}$=0,23; $\chi^2_{к-2}$=1,41; $p_{к-2}$=0,24; χ^2_{1-2}=0,001; p_{1-2}=0,97;

Note: statistical significance of differences between: $p_{к-1}$ — control group and group 1; $p_{к-2}$ — control group and group 2; p_{1-2} — between groups 1 and 2 of patients

Table 4. *Vitamin D concentration in carriers of different genetic polymorphisms of the vitamin D receptor gene VDR: 283 A>G (BsmI), VDR: 2 A>G (FokI)*

Gene	Genotypes	Concentration of 25(OH) D, ng/ml Me [Q1; Q3]	Statistics	
			Kruskal-Wallis	Manna-Whitney
				Comparison of study groups
VDR: 283 A>G	A/A n=29	26,2 [26,2; 28,3]	H=0,6 df=2 p=0,74	U_{1-2}=807,5, p_{1-2}=0,86; U_{1-3}=915,0 p_{1-3}=0,44; U_{2-3}=1763,0 p_{2-3}=0,26.
	A/G n=58	27,9 [27,3; 28,5]		
	G/G n=69	26,6 [26,6; 27,9]		
VDR:2 A>G	A/A n=35	26,0 [25,7; 27,1]	H=2,96 df=2 p=0,23	U_{1-2}=1011,5, p_{1-2}=0,98; U_{1-3}=1008,0, p_{1-3}=0,48; U_{2-3}=1741,5; p_{2-3}=0,66;
	A/G n=58	27,9 [27,5; 28,7]		
	G/G n=63	26,6 [26,6; 28,3]		

Note: p_{1-2} — statistical significance of differences between carriers of A/A polymorphism and A/G; p_{1-3} — statistical significance of differences between carriers of A/A polymorphism and G/G; p_{2-3} — statistical significance of differences between carriers of A/G polymorphism and G/G

No differences were found when studying vitamin D concentrations in patients with COVID-19-associated lung involvement, depending on polymorphism of vitamin D receptor gene VDR: 283 A>G (BsmI) and VDR: 2 A>G (FokI) (Table 4). Similar results were observed in other studies; for example, Smagina IV et al. studied patients with multiple sclerosis and found reduced serum concentrations of 25-hydroxyvitamin D25; however, there was no significant difference in plasma 25(OH)D levels in patients with various genotypes of these polymorphisms — 283 A>G (BsmI) and VDR: 2 A>G (FokI) [20].

Analysis of scientific literature on the study of genetic implications of the association between vitamin D deficiency and severity of the past COVID-19 infection shows ambiguous data; for instance, Shreiner EV, Petukhova SK, Khavkin AI et al. did not find out any association between the studied genotypes and severity of the past coronavirus infection [17]. At the same time, Protas VV et al. analysed information on various allele combinations of vitamin D receptor gene VDR: 2 A>G (FokI), including A>G, and summarised data on the association between such diseases as dengue fever, bronchopulmonary diseases (bronchial asthma, TB), Parkinson disease, and hepatitis B [11,21-25]. Analyses by Li, Qian MM and other foreign researchers mention some association between inheritance of certain polymorphisms of vitamin D receptor gene VDR, particularly rs1544410 (BsmI), rs 2228570 (FokI), and sepsis in various pathologies [26]. In their metaanalysis, Palshina AM et al. found out that in Caucasian patients in France, who carry FF frequency of genotype FokI, rheumatoid arthritis is much more common [27, 28]. A study of the Russian population of patients with arterial hypertension to analyse the distribution of genotypes FokI of VDR gene, encoding vitamin D receptor, demonstrated that in subjects with genotypes FokI FF and Ff of gene VDR, the disease onsets in younger age [27, 29]. A metaanlysis of VDR gene polymorphism, in particular of FokI and BsmI, in type 1 diabetes mellitus (79 studies) and type 2 diabetes mellitus (44 studies) showed a high risk of type 1 diabetes mellitus in the presence of allele B BsmI and type 2 diabetes mellitus in the presence of allele f FokI [27, 30]. In studies by Kostik MM et al., genotype bb of BsmI of a gene VDR polymorphous marker manifested as a marker of poor prognosis in boys with juvenile idiopathic arthritis [31].

Meanwhile, according to various estimates, the genotype contribution to fluctuation of serum 25(OH)D levels is 23–43 % to 77–80 % [32, 33]. If the patient's genotype has "risk alleles", i.e. genotype variants that cause a reduction in the amount or function of VDR receptors, vitamin D will not be fully absorbed by respective cells from the blood. A metabolic disorder will develop, similar to vitamin deficiency. At the same time, blood vitamin D level can remain normal. These genotypes account for 48 %, approximately 7–11 % of them have 2 "risk alleles" at once [32, 33].

Currently, only an incomplete list of genes, mutations of which impact vitamin D status, has been compiled [32]. Our idea of the genetic structure of 25(OH)D levels may be expanded as a result of large-scale genetic testing, analysis of "gene-gene" and "gene-environment" interactions, epigenetic observations, etc. Further studies in this area are likely to ensure better understanding of the mechanisms behind vitamin D metabolism regulation. How identified genetic polymorphisms affect vitamin D metabolism is not clearly known [32]. A majority of authors believe that serum 25(OH)D concentrations depend on both gene polymorphism and environmental factors (UV index, skin exposure to sunlight, nutritional path), therefore, they should be considered together.

Nevertheless, features of polymorphism of vitamin D receptor gene VDR can have an indirect impact on the function of innate and acquired immunity. It is essential to undertake a further study of the peculiarities of the genetic status of patients with COVID-19-associated lung involvement and search for haplotypes (including a study of cytokine gene polymorphisms, etc.) that affect disease severity, including sepsis.

Conclusions

Therefore, given the available results for patients with past COVID-19-associated lung involvement, it can be assumed that low blood levels of total 25-hydroxyvitamin D can contribute to a more severe course of coronavirus infection. Analysis of polymorphism of vitamin D receptor gene VDR:283 A>G demonstrated predominant inheritance of allele A and homozygote A/A in patients with a more severe disease, where pulmonary tissue involvement is over 50 % (CT-3, 4) associated with COVID-19 infection. Study of polymorphism of vitamin D receptor gene VDR: 2 A>G showed that most patients were homozygote A/A carriers vs. controls.

Вклад авторов:

Все авторы внесли существенный вклад в подготовку работы, прочли и одобрили финальную версию статьи перед публикацией

Караченова А.М.: вклад автора в разработку концепции и дизайна исследования, сбор, анализ и интерпретация данных, анализ литературы по теме исследования, научное редактирование, существенный вклад в научно-исследовательскую работу

Романова Е.Н.: вклад автора в разработку концепции и дизайна исследования, анализ литературы по теме исследования, научное редактирование, утверждение окончательного текста статьи, существенный вклад в научно-исследовательскую работу

Authors' contributions:

All authors made significant contributions to the preparation of the work, read and approved the final version of the article before publication

Karachenova A.M.: author's contribution to the development of the concept and design of the study, collection, analysis and interpretation of data, analysis of literature on the research topic, scientific editing, significant contribution to research work

Romanova E.N.: author's contribution to the development of the concept and design of the study, analysis of literature on the research topic, scientific editing, approval of the final text of the article, significant contribution to research work

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