Cellular and Molecular Biology
E-ISSN : 1165-158X / P-ISSN : 0145-5680
www.cellmolbiol.org

The vitamin D binding protein gene polymorphism association with Covid-19-infected Iraqi patients

Nawres Adnan Abdulameer[1,2*], Malek Eleroui[3], Hassan H. Nasser[4], Ibtissem Ben Amara[3]

[1] Department of Biology, Faculty of Science, University of Sfax, Tunisia
[2] Technical Institute of Al-Diwaniyah, Al-Furat Al-Awsat Technical University (ATU), Iraq
[3] Laboratory of Medicinal and Environment Chemistry, Higher Institute of Biotechnology, University of Sfax, BP 261, Sfax 3000, Tunisia
[4] Department of Microbiology, College of Veterinary Medicine, University of Al-Qadisiyah, Al-Qadisiyah, Iraq

ARTICLE INFO

Original paper

Article history:
Received: November 22, 2022
Accepted: April 14, 2023
Published: May 31, 2023

Keywords:

Covid-19, BPD, polymorphism and vitamin D.

ABSTRACT

Vitamin D deficiency, in general, is usually correlated with increased risks of chronic pulmonary disease such as COVID-19 infection. However, the mechanisms are still unknown. Herein, this study aimed to investigate the correlation between vitamin D binding protein gene polymorphism and COVID-19-infected Iraqi patients. The study has been conducted on patients with Covid-19 during the period extended from June, 2021 to April, 2022. 300 samples were collected from healthy and infected people. The demographic characteristics of patients (age, gender and residency) are shown non-significant in all. However, the distribution of *DBP (rs12785878*-T/G*)* Polymorphism was detected by Allele Specific PCR technique. The association between *DBP (rs12785878)* gene polymorphism and risk of Covid-19 is also shown, the heterozygous genotype TG was more frequent in the patients' group in comparison with the control group, 66 versus 58, respectively. Therefore, genotype TG was a genetic risk factor for Covid-19 with an odds ratio of 2.4074 (95% confidence interval of 1.2462-4.6505) and an etiologic fraction of 0.2963. In the addition, the homozygous genotype GG was more frequent in the patients' group in comparison with the control group, 65 versus 54, respectively, Therefore, genotype GG was a risk factor for Covid-19 with an odds ratio of 1.0578 (95% confidence interval of 0.6386-1.7522) and an etiologic fraction of 0.0299. thus, it can be seen that Covid-19 disease has a direct effect on the level of vitamin D in patients infected with the virus compared to healthy people.

Doi: http://dx.doi.org/10.14715/cmb/2023.69.5.5 Copyright: © 2023 by the C.M.B. Association. All rights reserved.

Introduction

Although coronavirus disease 2019 (COVID-19) is asymptomatic or has mild symptoms in the majority of the population, it may lead to death by causing serious clinical syndromes such as pneumonia, acute respiratory distress syndrome (ARDS), myocarditis, microvascular thrombosis, and cytokine storm in some patients (1). Extensive research has explored the effects of vitamin D on the treatment and complications of COVID-19 and its potential contribution to the reduction of COVID-19 incidence. Vitamin D exerts antiviral activity and inhibits viral replication by stimulating the release of cathelicidin and defensin proteins in monocytes and macrophages (2). Vitamin D plays an important role in preventing respiratory system infections due to its effects such as stimulating the chemotaxis of T-lymphocytes and clearing respiratory pathogens by inducing apoptosis and autophagy in the infected epithelium (3). It has been reported that low T-lymphocyte levels were found in some COVID-19 patients with severe symptoms (4). Since vitamin D supplementation increases the level of T-lymphocytes (5), this finding provides support for the hypothesis that vitamin D could be useful in the treatment of COVID-19. The severe progression of COVID-19 in some patients is one of the most important problems of the pandemic. Studies have pointed out an increased rate of thrombotic events and cytokine storm in severe COVID-19 patients. These events are responsible for fatal outcomes (6; 7; 8). Although the study of Faniyi et al. (9) suggested that vitamin D deficiency is an independent risk factor for the development of COVID-19 seroconversion, with the biggest differences in the BAME male group, a recent, not yet peer-reviewed Mendelian. A randomization study on vitamin D and COVID-19 susceptibility and severity in individuals of European ancestry, showed no protection of genetically increased 25(OH)D concentrations against COVID-19 susceptibility, hospitalization, or severe disease.

Materials and Methods

Samples collection

Samples were collected from Al-Diwaniyah Hospital from patients infected with Covid-19. The sample was divided into two parts, a section for DNA extraction to polymorphism and the other section for the purpose of examining the level of vitamin D.

Primers

ARMS-PCR primers for vitamin D binding protein gene polymorphism (DBP rs12785878 -T/G) were designed in this study using the NCBI-SNP data base and Primer1 ARMS-PCR primers design online. These primers were provided by (Scientific Researcher. Co. Ltd. Iraq) as

* Corresponding author. Email: dw.noras@atu.edu.iq

Table 1. The ARMS-PCR primers with their sequence and amplicon size.

Primer	Sequence (5'-3')	Product size
Wild type Forward Primer	CGTCTTTTGTGTTTAATTTCTTT	
Mutant Forward Primer	CGTCTTTTGTGTTTAATTTCTTC	515bp
Common Reverse Primer	CCAGTAAGAACAAGAGGCGGT	

follows in Table 1.

Statistical methods

All statistical analyses were performed using the IBM SPSS software package ver. 22. Chi-square test was used to compare the genotype SNPs between the patients with COVID-19 and healthy groups. In addition, the Hardy–Weinberg equilibrium for the three SNPs in the patients with COVID-19 and healthy groups was evaluated using the chi-square test. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated using logistic regression to evaluate the effects of these differences. P values of 0.05 or less were considered statistically significant.

Results

Demographic characteristics of patients and control subjects

The present study included 150 patients with Covid-19 and 150 apparently healthy subjects (Table 2). The demographic characteristics of patients and control subjects regarding age, gender and residency which is a prerequisite for such a case control study are shown in Table 2. The mean age of patients was (39.3 ± 16.16) and that of control subjects was (25.06 ± 12.12) years old and there was a non-significant difference at (P<0.05) between patients and control subjects in mean age. Also, according to age distribution, the frequency of patients and control showed non-significant differences at (P<0.05). The frequency distribution of patients and control subjects according to gender, patients group included 76 (50.66%) males and 74 (49.33 %) females, whereas, the control group included 72 (48.00 %) males and 78 (52.00 %) females and there was no significant difference at (P<0.05). According to residency, the patients group included 96 (64.00%) cases from urban areas and 54 (36.00%) cases from rural areas, while the control group included 88 (58.66%) cases from urban areas and 62 (41.33 %) cases from rural areas and there was no significant difference at (P<0.05) in the frequency distribution of patients and control subjects according to residency.

Socioeconomic and lifestyle variables

The present study indicates statistically no significant difference (P<0.05) between Covid-19 and occupation. As shown in Table 3, most Covid-19 patients were employments 28 (18.66%) compared with control 25 (16.66%). While Covid-19 patients were not employments 36 (24.00 %) compared with control 32 (21.33 %). And Covid-19 patients were student 24 (16.00 %) compared with control 22 (14.66%). Although Covid-19 patients were retired 17 (11.33%) compared with control 26 (17.33%). Covid-19 patients were housewives 20 (13.33 %) compared with control 19 (12.66%). Although Covid-19 patients were military 25 (16.66%) compared with control 26 (17.33 %). The frequency distribution of Covid-19 patients and healthy controls according to smoking showed Covid-19 increased in non-smoking groups in comparison to the smoking group, 99 (66.00%) versus 89 (59.33 %) respectively, but when compared to healthy controls indicate no significant association at (P<0.05) between smoking and Covid-19. The frequency distribution of Covid-19 patients and healthy controls according to married was as follows: 97 (64.66 %) patients were married and 53 (35.33 %)

Table 2. Demographic characteristics of patients and control subjects.

Characteristic	Patients	Control	*P-value*
Age (years)			
Mean ±SD	39.3 ± 16.16	25.06 ± 12.12	0.075 ¥
Range	10-60	12-55	NS
10-20, *n* (%)	18 (12.00%)	17 (11.33%)	
21-30, *n* (%)	32(21.33%)	39 (26.00%)	0.664 ¥
31-40, *n* (%)	42 (28.00%)	45 (30.00%)	NS
>41, *n* (%)	58 (38.66%)	49 (32.66%)	
Total *n* (%)	150(100.00%)	150 (100.00%)	
Gender			
Male, *n* (%)	76 (50.66%)	72 (48.00%)	
Female, *n* (%)	74 (49.33%)	78 (52.00%)	0.644 ¥
Total	150(100.00%)	150(100.00%)	NS
Male: Female	1:1.02	_	
Residency			
Urban, *n* (%)	96 (64.00 %)	88 (58.66%)	0.342 ¥
Rural, *n* (%)	54 (36.00%)	62 (41.33%)	NS
Total	150(100.00%)	150(100.00%)	

n: number of cases; **SD**: standard deviation; t-test; ¥: Chi-square test; HS: highly significant at P ≤ 0.05.

Table 3. Socioeconomic and lifestyle variables.

Characteristic	Patients	Control	P- value
Occupation			
Student, *n* (%)	24 (16.00%)	22 (14.66%)	
Employee, *n* (%)	28 (18.66%)	25 (16.66%)	
No employee, *n* (%)	36 (24.00%)	32 (21.33%)	0.788 ¥
Retired, n (%)	17 (11.33%)	26 (17.33%)	NS
Housewife, *n* (%)	20 (13.33%)	19 (12.66%)	
Military, *n* (%)	25 (16.66%)	26 (17.33%)	
Total, *n* (%)	150(100.00%)	150(100.00%)	
Smoking			
Yes, *n* (%)	99 (66.00%)	89 (59.33%)	0.232 ¥
No, *n* (%)	51 (34.00%)	61 (40.66%)	NS
Total, *n* (%)	150(100.00%)	150(100.00%)	
Married			
Yes, *n* (%)	97 (64.66%)	84(56.00%)	0.124 ¥
No, *n* (%)	53 (35.33%)	66 (44.00%)	NS
Total, *n* (%)	150(100.00%)	150(100.00%)	

n: number of cases; ¥: Chi-square test; S: significant; NS: Non-significant.

don't marry compared to healthy controls 84 (56.00%) were married and 66 (44.00 %) don't marry. The distribution of Covid-19 patients and healthy control in relation to socioeconomic and lifestyle variables are shown in Table 3.

Vitamin D Concentration (ng/ml) of patients and control subjects

The results of vitamin D Concentration (ng/ml) of patients and control subjects are shown in Table 4. There was a significant difference at ($P<0.05$) in the frequency distribution of patients and control subjects in vitamin D concentration. The Patient group was Vit. D concentration (4.78 ± 0.98), whereas, the control group was (37.09 ± 13.33).

Detection of *DBP (rs12785878*-T/G*)* Polymorphism

The distribution of *DBP (rs12785878*-T/G*)* Polymorphism was detected by Allele Specific PCR technique (Figures 1 and 2). At this SNP there are three genotypes; TT, GG and TG. The (TT) wild type homozygote was shown in the T allele only, the (GG) mutant type homozygote was shown in G allele only, whereas the (T/G) heterozygote was shown in both T and G alleles. The presence of the T or G allele was observed at 515bp product size (Figure 1). In the control group, the (TT) wild type homozygote was shown in the T allele only, the (GG) mutant type homozygote was shown in the G allele only, whereas the (T/G) heterozygote was shown in both T and G allele. The presence of the T or G allele was observed at 515bp product size.

Table 4. Vit. D Concentration (ng/ml) of patients and control subjects.

Groups	Patients	Control
Mean ± STD	4.78±0.98	37.09±13.33
Chi-Square (χ^2)	8.311	
P –Value	0.04 S	

Genotypic and Alleles Frequency Analysis for studied genes in Patients with Covid-19 and Control groups

The association between *DBP (rs12785878)* gene polymorphism and the risk of Covid-19 is shown in Table 5. The heterozygous genotype TG was more frequent in the patients group in comparison with the control group, 66

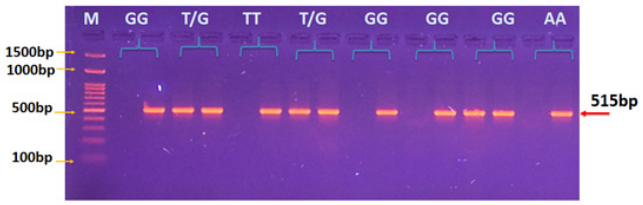

Figure 1. Agarose gel electrophoresis image that showed the Allele Specific PCR product analysis of vitamin D binding protein gene polymorphism (DBP rs12785878 -T/G) gene polymorphism in patient's samples. Where M: marker (2000-100bp). The (TT) wild type homozygote was shown in the T allele only, the (GG) mutant type homozygote were shown in G allele only, whereas the (T/G) heterozygote was showed in both T and G allele. The presence of the T or G allele was observed at 515bp product size.

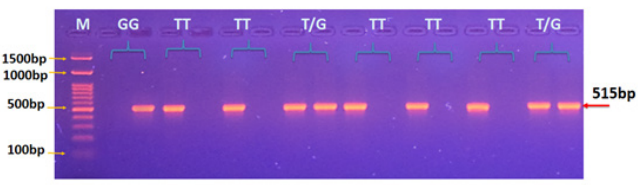

Figure 2. Agarose gel electrophoresis image that showed the Allele Specific PCR product analysis of vitamin D binding protein gene polymorphism (DBP rs12785878 -T/G) gene polymorphism in healthy control samples. Where M: marker (2000-100bp). The (TT) wild type homozygote was showed in the T allele only, the (GG) mutant type homozygote was shown in G allele only, whereas the (T/G) heterozygote was shown in both T and G alleles. The presence of the T or G allele was observed at 515bp product size.

Table 5. *DBP rs12785878 -T/G* POLY genotype frequency in patients with Covid-19 and control group.

DBP rs12785878	Patients n = 150	Control n =150	P1	P2	OR	95% CI	EF
GG	65(43.33%)	54(36.00%)	0.01958	0.0815 ¥ NS	1.0578	0.6386-1.7522	0.0299
TG	66(44.00%)	58(38.66%)	¥	0.00867 ¥ S	2.4074	1.2462-4.6505	0.2963
TT	19(12.66%)	38(25.33%)	S	Reference	Reference	Reference	Reference

***P*1**: overall comparison; ***P*2**: Individual genotype comparison versus reference; **n:** number of cases; ¥: Chi-square test; OR: odds ratio; **CI**: confidence interval; **EF**: etiologic fraction; NS: non-significant.

versus 58, respectively and the difference was non-significant at (P<0.05). Therefore, genotype TG was a genetic risk factor for Covid-19 with an odds ratio of 2.4074 (95% confidence interval of 1.2462-4.6505) and an etiologic fraction of 0.2963. On the other hand, the homozygous genotype GG was more frequent in patients group in comparison with the control group, 65 versus 54, respectively, but the difference was significant at (P<0.05). Therefore, genotype GG was a risk factor for Covid-19 with an odds ratio of 1.0578 (95% confidence interval of 0.6386-1.7522) and an etiologic fraction of 0.0299.

Discussion

This study investigated the relationship between vitamin D binding protein gene polymorphism and COVID-19-infected Iraqi patients. As the COVID-19 pandemic spread over the world, it severely disrupted people's daily lives. The pathogenesis of COVID-19 disease has been linked to several physiological processes and signalling systems, including vitamin D (10). Its importance cannot be overstated due to vitamin D's ability to fight off bacteria and control the immune system (11). As a result, this study confirms the findings of previous research that found a correlation between vitamin D and COVID-19. Vitamin D deficiency has been linked to poor outcomes, and reports of its prevalence are exceptionally high in COVID-19 patients in severe conditions. Despite the link between vitamin D deficiency and severe COVID-19, there is still some debate as to whether or not vitamin D can protect against the disease due to its impact on adaptive and innate immunity. Recent research has linked vitamin D deficiency to COVID-19 disease, and investigations have shown a correlation between pneumonia and low serum levels of 25-hydroxyvitamin D (12). The study's objective is to link specific variations in vitamin D receptor genes to the COVID-19 virus. DBP is the primary molecule in charge of transporting and storing vitamin D metabolites in the blood. DBP binds roughly 85% of vitamin D metabolites in healthy individuals, while albumin binds only about 15% (13). Many polymorphisms in the vitamin D metabolic pathway work together to dramatically affect its levels and effects, which may explain this phenomenon. Polymorphisms in genes like vitamin D binding protein (DBP) that are part of the vitamin D metabolic pathway have been linked to the development and spread of COVID-19, and this work adds to the expanding body of evidence supporting this hypothesis. The presence of distinct polymorphisms in each study population may account, at least in part, for the divergent findings concerning vitamin D and COVID-19 (14). Vitamin D supplement efficacy can be diminished by environmental influences and changes in DBP and other genes involved in vitamin D metabolism. Al-Horani et al.(15) investigated common DBP polymorphisms in vitamin D supplementation. Study participants with the significant homozygous rs7041 genotype had much higher 25[OH]D levels than those with other genotypes, and those with the homozygous rs4588 genotype had the highest levels of all. The wide variety of genetic polymorphisms suggests that the optimal vitamin D level may vary depending on where to lives. These potential explanations should be considered in future studies examining the effect of vitamin D on genetic diversity in vitamin D metabolism among populations (10). Research on adolescents with COVID-19 found that serum vitamin D levels were related to clinical severity, inflammatory markers, and lymphocyte count. These associations held more valid in the absence of vitamin D than in the presence of sufficient levels (16). Significantly higher levels of vitamin D insufficiency were observed over the COVID-19 severity range (p 0.001). A retrospective observational study conducted found that people with high COVID-19 severity were likelier to have low vitamin D levels (14). Vitamin D insufficiency is a worldwide epidemic, according to Alshahawey (17). Preventative vitamin D supplementation is standard in several countries, especially for young children and adolescents. Generally speaking, vitamin D supplements are only provided to infants in the United States. Only 18.4 percent of this population had sufficient levels of 25-hydroxyvitamin D3. Adolescents' vitamin D levels were often lower than younger children or adults. The high rates of vitamin D insufficiency and the deficiency that coincided with the end of winter in our country may be explained by the low sun exposure from quarantine at the onset of the pandemic (12). Due to the widespread usage of vitamin D supplements in children younger than one-year-old, neonatal infants were also excluded. Preventative vitamin D supplementation should be considered a public health precaution during the COVID-19 pandemic because of the high frequency of vitamin D insufficiency and inadequacy among adolescents (16). Although COVID-19 is a global epidemic, the results of this single-centre study should be regarded with caution since they may not apply to persons in other countries. There is evidence that differences in ACE2 or vitamin D receptor genes contribute to illness susceptibility or severity (18). However, researchers discovered that the TT genotype significantly increased the likelihood of developing COVID-19. Researchers have observed that the GT genotype increases population vulnerability to this disease. The observed heterogeneity in COVID-19 mortality rates among nations may have origins in differences in vitamin D metabolism caused by the rs7041 and rs451 DBP polymorphisms (12; 19; 20). The free vitamin D metabolite levels responsible for DBP are tightly regulated in various clinical settings, making DBP the most changeable protein known. The affinity of individual DBP alleles for vitamin D metabolites varies, which can have significant implica-

tions for various diseases and disorders (21). Multiple populations in the human population have been shown to have genetic variations in the vitamin D binding protein (DBP) gene that contribute to vitamin D insufficiency. Researchers estimate that 7 and 77 per cent of people worldwide do not get enough vitamin D. Compared to tropical and high-latitude regions, subtropical and mid-latitude areas have a higher prevalence of vitamin D insufficiency (22). Average blood 25(OH)D levels were 16.61 ng/mL across all age groups in a study of 1,161 healthy Turks. Vitamin D insufficiency was also demonstrated, with results showing that 75.54 per cent of the adult population in Turkey has 25(OH)D levels below 20 ng/mL. Genetics appears to play a significant influence in regulating vitamin D levels in both healthy and ill persons. This article focuses on one specific SNP in the DBP gene: rs7041 (23). Another small study found that for patients hospitalised with COVID-19 disease, high vitamin D dosages lowered the chance of admission to the critical care unit. Multiple clinical investigations have demonstrated that vitamin D supplementation reduces the severity and fatality rate of viral infections (19). According to Freitas et al. (10), 64% of the COVID-19 participants had severe symptoms, 59% had intermediate symptoms, and 76% had vitamin D deficiency at the time of death. Another study; found that those who took vitamin D supplements regularly for a year prior to contracting COVID-19 had a lower risk of developing severe illness and a better chance of surviving. These results support the study's preliminary conclusion that vitamin D and COVID-19 disease are associated with one another (18). In 20 European countries, Abrishami et al. (24) sought to determine if higher vitamin D levels in the blood were associated with decreased COVID-19-related mortality rates. This research established a correlation between the mean blood content of vitamin D and the incidence and mortality rates across countries. It has been observed that patients who are more likely to have vitamin D insufficiencies, such as the elderly and those of black and minority ethnic (BAME) ancestry, are at high risk for severe COVID-19 (11). Vitamin D insufficiency is more common in infants and children than adults, but the symptoms are fewer in young children and infants. This research aimed to measure serum 25(OH)D levels from the earliest stages of the disease in both inpatient and outpatient COVID-19 patients. Multiple studies are being undertaken, but no one has yet looked at whether or not vitamin D affects COVID-19 (12). It is fascinating to think about the possible explanations for the correlation between vitamin D and DBP/COVID-19. Clinical investigations involving administering various types of vitamin D to hospitalised patients with COVID-19 have shown conflicting results, suggesting that the immunomodulatory effects of vitamin D, if clinically meaningful, are more likely to be sustained than transient. Preventing vitamin D deficiency and the diseases it can cause may have more immediate effects than treating it (25). DBP SNPs, like vitamin D levels, have been linked to COVID-19 prevalence, mortality, and other chronic conditions. Speeckaert et al. (14) discovered that the existence of the DBP gene polymorphism (rs7041) was strongly linked with the incidence and death rates of COVID-19 in 55 countries, expanding on the work of Batur et al. (26). However, the ecological fallacy may be at play when combining data from different countries, so caution is warranted when extrapolating from this finding. Additional factors, such as comorbidities, may link DBP polymorphism and COVID-19. Specifically, we found a strong correlation between vitamin D status and COVID-19 outcomes for patients older than 65 with a body mass index of less than 30 kg/m2. As revealed by Speeckaert et al. (14), DBP's alleged causal relationship is intriguing. More research is needed into the association between COVID-19 and DBP polymorphisms, DBP levels, and total and free 25-hydroxyvitamin D. Batur et al. (26), which looked at the connection between vitamin D binding protein (DBP) gene polymorphisms and COVID-19 incidence and mortality, piqued our curiosity. There was a positive correlation between the GT genotype and the prevalence and death rates related to COVID-19, while there was a negative correlation between the TT genotype and the same factors. There was no evidence that the rs4588 polymorphism increased the danger of getting sick or dying. The rs7041 and rs452 DBP polymorphisms control vitamin D metabolism, and the authors hypothesise that this may explain why susceptibility to and mortality from COVID-19 vary throughout the nations examined (26,27). Although the TT genotype is less frequent than the GG and GT genotypes, previous investigations in healthy white premenopausal women have connected it to lower plasma 25-hydroxyvitamin D concentrations (11).

References

1. Cascella M, Rajnik M, Cuomo A, et al. Features, evaluation and treatment coronavirus (COVID-19). Stat Pearls. Treasure Island (FL): Stat Pearls Publishing, 18 May 2020.
2. Gombart AF, Borregaard N, Koeffler HP. Human cathelicidin antimicrobial peptide (CAMP) gene is a direct target of the vitamin D receptor and is strongly up-regulated in myeloid cells by 1,25-dihydroxyvitamin D3. FASEB J. 2005 Jul;19(9):1067-77. doi: 10.1096/fj.04-3284com. PMID: 15985530.
3. Adams JS, Sharma OP, Gacad MA, Singer FR. Metabolism of 25-hydroxyvitamin D3 by cultured pulmonary alveolar macrophages in sarcoidosis. J Clin Invest. 2009;72(5):1856–60.
4. Chen L, Eapen MS, Zosky GR. Vitamin D both facilitates and attenuates the cellular response to lipopolysaccharide. Sci Rep 2017;7:45172.
5. Cantorna MT. Ooi JH, McDaniel KL, Weaver V, Murine CD8+ T cells but not macrophages express the vitamin D 1alpha-hydroxylase. J Nutr Biochem 2015;25(1):58–65.
6. Di Santo SG, Franchini F, Filiputti B, Martone A, Sannino S. The Effects of COVID-19 and Quarantine Measures on the Lifestyles and Mental Health of People Over 60 at Increased Risk of Dementia. Front Psychiatry. 2020 Oct 14;11:578628. doi: 10.3389/fpsyt.2020.578628. PMID: 33173523; PMCID: PMC7591702.
7. Hu B, Guo H, Zhou P, Shi ZL. Characteristics of SARS-CoV-2 and COVID-19. Nat Rev Microbiol. 2021 Mar;19(3):141-154. doi: 10.1038/s41579-020-00459-7. Epub 2020 Oct 6. Erratum in: Nat Rev Microbiol. 2022 May;20(5):315. PMID: 33024307; PMCID: PMC7537588.
8. Wang C, Horby PW, Hayden FG, Gao GF. A novel coronavirus outbreak of global health concern. Lancet. 2020 Feb 15;395(10223):470-473. doi: 10.1016/S0140-6736(20)30185-9. Epub 2020 Jan 24. Erratum in: Lancet. 2020 Jan 29;: PMID: 31986257; PMCID: PMC7135038.
9. Faniyi AA, Lugg ST, Faustini SE, Webster C, Duffy JE, Hewison M, Shields A, Nightingale P, Richter AG, Thickett DR. Vitamin D status and seroconversion for COVID-19 in UK healthcare workers. Eur Respir J. 2021 Apr 8;57(4):2004234. doi:

10.1183/13993003.04234-2020. PMID: 33303541; PMCID: PMC7736751.

10. Freitas AT, Calhau C, Antunes G, Araújo B, Bandeira M, Barreira S, Bazenga F, Braz S, Caldeira D, Santos SCR, Faria A, Faria D, Fraga M, Nogueira-Garcia B, Gonçalves L, Kovalchuk P, Lacerda L, Lopes H, Luís D, Medeiros F, Melo AMP, Melo-Cristino J, Miranda A, Pereira C, Pinto AT, Pinto J, Proença H, Ramos A, Rato JPR, Rocha F, Rocha JC, Moreira-Rosário A, Vazão H, Volovetska Y, Guimarães JT, Pinto FJ. Vitamin D-related polymorphisms and vitamin D levels as risk biomarkers of COVID-19 disease severity. Sci Rep. 2021 Oct 21;11(1):20837. doi: 10.1038/s41598-021-99952-z.
11. Hafez W, Saleh H, Arya A, Alzouhbi M, Fdl Alla O, Lal K, Kishk S, Ali S, Raghu S, Elgaili W, Abdul Hadi W. Vitamin D Status in Relation to the Clinical Outcome of Hospitalized COVID-19 Patients. Front Med (Lausanne). 2022 Mar 29;9:843737. doi: 10.3389/fmed.2022.843737. PMID: 35425774; PMCID: PMC9004341.
12. Hashemi, S. M. A., Thijssen, M., Hosseini, S. Y., Tabarraei, A., Pourkarim, M. R., & Sarvari, J. Human gene polymorphisms and their possible impact on the clinical outcome of SARS-CoV-2 infection. Arch Virol 2021; 166(8): 2089–2108. https://doi.org/10.1007/s00705-021-05070-6
13. Carpagnano GE, Di Lecce V, Quaranta VN, Zito A, Buonamico E, Capozza E, Palumbo A, Di Gioia G, Valerio VN, Resta O. Vitamin D deficiency as a predictor of poor prognosis in patients with acute respiratory failure due to COVID-19. J Endocrinol Invest. 2021 Apr;44(4):765-771. doi: 10.1007/s40618-020-01370-x. Epub 2020 Aug 9. PMID: 32772324; PMCID: PMC7415009.
14. Speeckaert MM, Delanghe JR. Vitamin D binding protein and its polymorphisms may explain the link between vitamin D deficiency and COVID-19. Sci Prog. 2021 Oct;104(4):368504211053510. doi: 10.1177/00368504211053510. PMID: 34723751.
15. Al-Horani H, Abu Dayyih W, Mallah E, Hamad M, Mima M, Awad R, Arafat T. Nationality, Gender, Age, and Body Mass Index Influences on Vitamin D Concentration among Elderly Patients and Young Iraqi and Jordanian in Jordan. Biochem Res Int. 2016;2016:8920503. doi: 10.1155/2016/8920503. Epub 2016 Mar 24. PMID: 27110402; PMCID: PMC4823503.
16. Newton DA, Baatz JE, Kindy MS, Gattoni-Celli S, Shary JR, Hollis BW, Wagner CL. Vitamin D binding protein polymorphisms significantly impact vitamin D status in children. Pediatr Res. 2019 Nov;86(5):662-669. doi: 10.1038/s41390-019-0322-y. Epub 2019 Feb 2. PMID: 30712059; PMCID: PMC6677641.
17. Alshahawey M. A genetic insight into vitamin D binding protein and COVID-19. Med Hypotheses. 2021 Apr;149:110531. doi: 10.1016/j.mehy.2021.110531. Epub 2021 Feb 9. PMID: 33607405; PMCID: PMC7871104.
18. Mamurova B, Akan G, Mogol E, Turgay A, Tuncel G, Evren EU, Evren H, Suer K, Sanlidag T, Ergoren MC. Strong Association between Vitamin D Receptor Gene and Severe Acute Respiratory Syndrome coronavirus 2 Infectious Variants. Glob Med Genet. 2023 Feb 16;10(1):27-33. doi: 10.1055/s-0043-1761924. PMID: 36819669; PMCID: PMC9935054.
19. Manca A, Cosma S, Palermiti A, Costanzo M, Antonucci M, De Vivo ED, Ianniello A, Borella F, Carosso AR, Corcione S, De Rosa FG, Benedetto C, D'Avolio A, Cusato J. Pregnancy and COVID-19: The Possible Contribution of Vitamin D. Nutrients. 2022 Aug 10;14(16):3275. doi: 10.3390/nu14163275. PMID: 36014781; PMCID: PMC9414046.
20. Nasser-Dhabaan, A., & Alwan, M. Polymorphisms of IFN-γ T/A +874 Gene and Relationship with COVID 19 in Iraqi Population. IOP Conference Series: Earth and Environmental Science 2021;790, 012049. https://doi.org/10.1088/1755-1315/790/1/012049
21. Ghazy, M. A genetic insight into vitamin D binding protein and COVID-19. Medical Hypotheses, (2021).149, 110531. https://doi.org/10.1016/j.mehy.2021.110531
22. Bikle DD, Schwartz J. Vitamin D Binding Protein, Total and Free Vitamin D Levels in Different Physiological and Pathophysiological Conditions. Front Endocrinol (Lausanne). 2019 May 28;10:317. doi: 10.3389/fendo.2019.00317. PMID: 31191450; PMCID: PMC6546814.
23. Apaydin, T., Polat, H., Dincer Yazan, C., Ilgin, C., Elbasan, O., Dashdamirova, S., Bayram, F., Tukenmez Tigen, E., Unlu, O., Tekin, A. F., Arslan, E., Yilmaz, I., Haklar, G., Ata, P., & Gozu, H. Effects of vitamin D receptor gene polymorphisms on the prognosis of COVID-19. Clinical Endocrinology, (2022). 96(6), 819–830. https://doi.org/10.1111/cen.14664
24. Abrishami, A., Dalili, N., Mohammadi Torbati, P., Asgari, R., Arab-Ahmadi, M., Behnam, B., & Sanei-Taheri, M. Possible association of vitamin D status with lung involvement and outcome in patients with COVID-19: A retrospective study. Eur J Nut 2021;60(4): 2249–2257. https://doi.org/10.1007/s00394-020-02411-0
25. Speeckaert MM, Delanghe JR. Contribution of Vitamin D-Binding Protein Polymorphism to Susceptibility and Outcome of COVID-19 Patients. J Nutr. 2021 Aug 7;151(8):2498-2499. doi: 10.1093/jn/nxab234. PMID: 34363485; PMCID: PMC8385920.
26. Zrieq R, Snoussi M, Algahtan FD, Tasleem M, Saeed M, Noumi E, Khalifa NE, Gad-Elkareem MAM, Aouadi K, Kadri A. Repurposing of anisomycin and oleandomycin as a potential anti-(SARS-CoV-2) virus targeting key enzymes using virtual computational approaches. Cell Mol Biol (Noisy-le-grand). 2022 Feb 4;67(5):387-398. doi: 10.14715/cmb/2021.67.5.51. PMID: 35818229.
27. Karcıoğlu Batur L, Özaydın A, Maviş ME, Gürsu GG, Harbige L, Hekim N. Vitamin-D Binding Protein Gene Polymorphisms and Serum 25-Hydroxyvitamin-D in a Turkish Population. Metabolites. 2021 Oct 12;11(10):696. doi: 10.3390/metabo11100696. PMID: 34677411; PMCID: PMC8537126.