A strong association between the VDR gene markers and SARS-CoV-2 variants

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Abstract

Introduction

A COVID-19 disease, caused by the SARS-CoV-2, created significant concern since December 2019 worldwide. The virus is known to be highly transmissible. Heterogenic clinical features even vary more among SARS-CoV-2 variants from asymptomatic forms to severe symptoms. Previous studies revealed an association between COVID-19 and vitamin D deficiency resulting from its low levels in COVID-19 patients. To our knowledge, there is no scientific investigation that evaluates the direct association between SARS-CoV-2 variants of concern and *VDR* gene markers in Cyprus. Thus, the present study aimed to identify the putative impact of *VDR* gene polymorphisms on SARS-CoV-2 infection among different variants.

Methods

The nasopharyngeal swabs were taken from a total number of 600 patients who were admitted to Near East University Hospital COVID-19 PCR Diagnosis Laboratory for routine SARS-CoV-2 RT-qPCR test. The RT-qPCR negative resulting samples were taken as control samples (n = 300). On the other hand, the case group consisted of patients who were SARS-CoV-2 RT-qPCR positive, infected with either SARS-CoV-2 Alpha (n = 100), Delta (n = 100), or Omicron (n = 100) variants. Two *VDR* gene polymorphisms, *Taq*I-rs731236 T > C and *Fok*I-rs10735810 C > T, were genotyped by PCR-RFLP.

Results

The mean age of the COVID-19 patient's ± SD was 46.12 ± 12.36 and 45.25 ± 12.71 years old for the control group ($p > 0.05$). The gender distribution of the patient group was 48.3% female and 51.7% male and for the control group 43% female and 57% male ($p > 0.05$). Significant differences were observed in genotype frequencies of *FokI* and *TaqI* variants between SARS-CoV-2 patients compared to the control group ($p < 0.005$). Furthermore, the risk alleles, *FokI* T allele and *TaqI* C, were found to be statistically significant (OR = 1.80, 95% CI = 1.42–2.29, OR = 1.62, 95% CI = 1.27–2.05, respectively) in COVID-19 patients. The highest number of patients with wild-type genotype was found in the control group, which is 52.9% compared with 17.5% in the case group. Moreover, most of the COVID-19 patients had heterozygous/homozygous genotypes, reaching 82.5%, while 47.1% of the control group patients had heterozygous/homozygous genotypes.

Conclusion

Our results suggested that patients with *FokI* and *TaqI* polymorphisms might tend to be more susceptible to getting infected with the SARS-CoV-2. Overall, findings from this study provided evidence regarding vitamin D supplements recommendation in individuals with vitamin D deficiency/insufficiency in the peri- or post-COVID-19 pandemic.

Introduction

A recent new coronavirus disease, COVID-19 pandemic, caused over 6.2 million deaths worldwide and still is a global health problem (Rauf et al., 2020; WHO, 2022). Viruses naturally adapt to environmental conditions over time. Therefore, viral variants might be raised due to mutations within the viral genome. These viral changes may allow the virus to spread more quickly and potentially trigger more serious complications (Raman et al., 2021). The SARS-CoV-2 genome has changed over time due to random mutations, its own recombination errors and survival adaptation, resulting in the emergence of genetic variants that are believed to be highly infectious (Safari and Elahi, 2022).

During the pandemic, the World Health Organization (WHO) declared SARS-CoV-2 variants of concern (VoC), which included Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), Delta (B.1.617.2), and Omicron (B.1.1.529). VoCs have a dramatically intensified attachment attraction in the receptor-binding domain – human Angiotensin Converting Enzyme 2 (RBD-hACE2) complex due to mutations in the spike (S) protein receptor-binding domain (RBD), enhancing the virus transmissibility (Shahhosseini et al., 2021). Moreover, VoCs may modulate or even enhance their ability to replicate regardless of growing immunity in the population, which could be occurred *via* infection recovery or vaccines (Hendy et al., 2021). However, Alpha and Delta variants were presented to be more contagious and caused more deaths among the other viral genomic make-ups (Gallagher J, 2021).

The interaction between human genomic variations and the COVID-19 disease severity still is unclear (Yi et al., 2020). Early studies indicated an association between COVID-19 disease and vitamin D deficiency (Carpagnano et al., 2021; Weir et al., 2020).

A steroid hormone, vitamin D, is a fat-soluble molecule that plays a vital role in bone strengthening, muscles, and the general well-being of individuals. The primary biological function of vitamin D is to keep average calcium and phosphorus blood levels (Krawiec and Dominiak, 2018). Vitamin D also has anti-inflammatory functions, especially in viral infections, and can act as an immune modulator (Charoenngam and Holick, 2020). Moreover, multiple studies have found that vitamin D has a wide range of effects on cell growth, differentiation, cell death, immunomodulation, and genome stability. Recent research has also identified a correlation between vitamin D and cardiovascular disease, diabetes, cancer, autoimmune diseases, and infectious diseases, which means that vitamin D deficiency is associated with a broad range of diseases (Wang et al., 2017).

Limited sun exposure, advanced age, a sedentary lifestyle, and vitamin D receptor *(VDR)* gene polymorphisms might be the factors linked to a greater risk of vitamin D deficiency (Kandemis et al., 2021). However, a recent study showed that the young Turkish Cypriots population in Northern Cyprus was found to be vitamin D deficient, despite the fact that the Island of Cyprus receives constant sunlight for almost nine months a year, and the Mediterranean diet is rich in vitamin D-containing foods (Kandemis et al., 2021; Tuncel et al., 2019).

Moreover, the polymorphisms on the *VDR* gene included the most well-known *TaqI*-rs731236 T > C, *ApaI*-rs7975232 A > C, *BsmI*-rs1544410 G > A, and *FokI*-rs10735810 C > T, which were associated with secretion of vitamin D and a higher risk of chronic diseases like autoimmune diseases, type 2 diabetes, and cancer (Li et al., 2013; Lee et al., 2011).

Studies on the associations of COVID-19 prevalence and mortality rates and genetic variability of the hosts showed that *VDR* gene polymorphisms may play role in modulating of COVID-19 infection and might be related to survival of the patients and COVID-19 severity (Abdollahzadeh et al., 2021; Al-Anouti et al., 2021). Therefore, *TaqI* and *FokI* gene polymorphisms might be contributed to modulating the response to vitamin D supplementation as linked to the improved reaction to supplementation as well as a predisposition to chronic diseases. Thus, this study aimed to investigate the possible impact of *VDR* gene markers on different SARS-CoV-2 infectious variants that caused long-pandemic.

Materials And Methods

Sample collection

The nasopharyngeal swabs were taken from a total number of 600 patients who were admitted to Near East University Hospital COVID-19 PCR Diagnosis Laboratory for routine SARS-CoV-2 RT-qPCR test. RT-qPCR was performed for possible SARS-CoV-2 detection according to the manufacturer's guidelines (Uniplex RT-qPCR SARS-CoV-2 RT-qPCR Detection Kit, IKAS Medical, Nicosia, Northern Cyprus). The RT-qPCR negative resulting samples were taken as control samples (n = 300). On the other hand, the case group consisted of patients who were SARS-CoV-2 RT-qPCR positive, infected with either SARS-CoV-2 Alpha (n = 100), Delta (n = 100), or Omicron (n = 100) variants.

SARS-CoV-2 variant identification by mutation typing

The SARS-CoV-2 variant analysis was performed by the Multiplex SARS-CoV-2 VoC RT-qPCR Detection Kit (IKAS Medical, Nicosia, Northern Cyprus). The mutations including del69/70, N501Y, K417N, T478K, Y144del, and P681R were used to identify B.1.1.7 (Alpha), B.1.351 (Beta), P.1 (Gamma), B.1.617.2 (Delta), B.1.1.529 (Omicron) variants. Delta variant was identified as follows: positive for 478K and P681R mutations and negative for the mutation H69-70 deletion, N501Y, K417N, and Y144del mutations. Omicron (BA.1) variant was identified as follows: positive for the H69-70 deletion, N501Y, T478K, K417N, and Y144del mutations, and negative for the P681R mutation. Lastly, the Alpha variant was identified as follows: positive for H69-70 deletion, N501Y, and Y144del mutations and negatives for T478K, K417N, and P681R mutations. All identified variants have been confirmed using Whole-genome sequencing.

VDR Gene Polymorphisms Genotyping

The human genomic DNA was extracted from volunteered SARS-CoV-2 RT-qPCR positive and negative (control) cases using Uniplex RT-qPCR SARS-CoV-2 RT-qPCR Detection Kit (IKAS Medical, Nicosia, Northern Cyprus). Two *VDR* gene polymorphisms (*Taq*I-rs731236 and *Fok*I-rs10735810) were genotyped by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Genotypes were

determined according to the presence and absence of the two restriction sites that were analyzed and alleles were designated with respect to actual base change according to the Ensembl (http://www.ensembl.org/) genome browser and NCBI SNP database (https://www.ncbi.nlm.nih.gov/SNP/, dbSNP).

Statistical Analysis

SPSS software (Statistical Package for the Social Sciences, SPSS Inc, Chicago, IL, USA, and version 25) was used to perform a statistical analysis of the data. Descriptive data and genotype data of the study group were demonstrated as mean ± standard deviation (SD) or number and frequency, where applicable. The Student's t-test and Mann–Whitney U test were used to make comparisons between normal and non-normally distributed quantitative variables. Chi-square (x2) was used to compare the genotype and allelic frequency distributions of *FokI* and *TaqI* polymorphisms between study groups. When the conditions for using the chi-square (X^2) test were not accomplished, Pearson's chi-square test or Fisher's exact test were used to confirm the association of categorical variables between study groups. Hardy-Weinberg equilibrium (HWE) was evaluated by Fischer's exact test. The OR and 95% CI were calculated using binary logistic regression analysis with co-dominant, dominant, additive, and recessive inheritance models. Akaike's information criterion (ACI) was used to select the inheritance model. To assess group differences, the data were log-transformed to satisfy ANOVA criteria before being subjected to one-way ANOVA with Tukey's posthoc analysis. *TaqI* and *FokI* polymorphisms were assessed for their relative risks in SARS-CoV-2 variants infected individuals by estimating odds ratios (ORs) and 95% confidence intervals (CIs), which were considered separate outcomes. In all cases, differences were found important at $p < 0.05$.

Results

In this study, we investigated the allelic frequencies and genotypic distribution between vitamin D receptor (*VDR*) gene *FokI* rs10735810 C > T and *TaqI* rs731236 T > C polymorphisms among patients infected by different SARS-CoV-2 VoC and compared with SARS-CoV-2 RT-qPCR negative subjects as a control group.

The study group included 300 COVID-19 patients who were infected by the SARS-CoV-2 Delta, Alpha, and Omicron BA.1 variants and 300 non-infected subjects as a control group. The mean age of the COVID-19 patient's ± SD was 46.12 ± 12.36 and 45.25 ± 12.71 years old for the control group ($p > 0.05$). The gender distribution of the patient group was 48.3% female and 51.7% male and for the control group 43% female and 57% male ($p > 0.05$).

Allelic and genotypic distribution frequency of VDR FokI and TaqI polymorphisms in the studied population

The genotypic distributions and allelic frequencies of *FokI* rs10735810 and *TaqI* rs731236 markers in COVID-19 patients and the control group were presented in Table 1. Significant differences were observed in genotype frequencies of *FokI* and *TaqI* variants between SARS-CoV-2 infected patients and the control group ($p < 0.005$) (Table 1).

Furthermore, the risk alleles, *FokI* T allele and *TaqI* C, were found to be statistically significant (OR = 1.80, 95% CI = 1.42–2.29, OR = 1.62, 95% CI = 1.27–2.05, respectively) in COVID-19 patients.

We also investigated genotypic distributions of *FokI* and *TaqI* polymorphisms together among the studied groups (case and control). The genotypes were grouped as follows; Group1: *FokI* TT (Wild Type) + *TaqI* TT (Wild Type), Group2: *FokI* TC + CC (Heterozygote + Homozygote) + *TaqI* TC + CC (Heterozygote + Homozygote).

Thus, the highest number of patients with group 1 (wild-type genotype) was found in the control group, which is 52.9% compared with 17.5% in the case group. Moreover, most of the COVID-19 patients had group 2 (heterozygous/homozygous genotypes), reaching 82.5%, while 47.1% of the control group patients had group 2 (Fig. 1).

These results suggested that patients with *FokI* and *TaqI* polymorphisms might tend to be more susceptible to getting infected with the SARS-CoV-2.

VDR gene polymorphism distributions among SARS-CoV-2 variants

Mutation analysis showed that *FokI* C > T polymorphism was distributed in at least one mutant allele (T) in 69% of SARS-CoV-2 Delta variant infected patients, while 54% of patients with SARS-CoV-2 Alpha variant infected with at least one mutant allele. Additionally, 77% of SARS-CoV-2 Omicron BA.1 variant infected patients carried at least one mutant allele for the same mutation (Fig. 2A). The differences in the distribution of the *FokI* polymorphism between the three variant groups were found as statistically significant ($p < 0.005$).

Furthermore, *TaqI* T > C polymorphism was distributed in 67% of SARS-CoV-2 Delta variant subjects with at least one altered allele, while 60% of SARS-CoV-2 Alpha variant subjects carried at least one mutant allele. Also, 72% of the SARS-CoV-2 Omicron BA.1 variant infected patients carried at least one mutant allele (Fig. 2B). However, the genotype distribution for *TaqI rs731236* polymorphism among patients who were infected with different SARS-CoV-2 variants did not show any statistical significance.

Analysis of VDR gene polymorphisms based on genetic inheritance models

In genetic association studies, statistical power to detect disease susceptibility loci depended on the genetic models tested. Therefore, the genotype frequencies were further analyzed by four genetic models:

additive, co-dominant, dominant, and recessive models (Table 2). For *TaqI*, a significant association between this polymorphism and increased risk of COVID-19 patients were found in all four models, co-dominant genotype (TT) vs (CC) (OR = 2.66, 95% CI = 1.59–4.47, p = 0.001); co-dominant genotype (CC) vs (TT) (OR = 0.37, 95% CI = 0.25–0.72, p = 0.001); dominant (OR = 2.28, 95% CI = 1.64–3.18, p = 0.001); recessive (OR = 0.55, 95% CI = 0.34–0.90, p = 0.016); additive (OR = 0.56, 95% CI = 0.44–0.71, p = 0.001). Moreover, significant positive correlations between *TaqI* and risk of COVID-19 patients were also identified in co-dominant genotype (TT) vs (CC) (OR = 2.32, 95% CI = 1.38–3.92, p = 0.001); co-dominant genotype (CC) vs (TT) (OR = 0.43, 95% CI = 0.25–0.72, p = 0.001); dominant (OR = 1.97, 95% CI = 1.41–2.73, p = 0.005); recessive (OR = 0.60, 95% CI = 0.36–0.97, p = 0.03); additive (OR = 0.61, 95% CI = 0.48–0.78, p = 0.001).

Discussion

COVID-19 disease created significant life concerns as a pandemic. The immune defenses of each patient were critical in limiting the risk of SARS-CoV-2 infection. COVID-19 pathophysiology might involve several signaling pathways and cellular components, including vitamin D2 (Raoult et al., 2020). Vitamin D is an essential immune system modulator and has an anti-infective and immunomodulatory effect (Aranow, 2011). Recent studies indicated an association between COVID-19 disease and vitamin D deficiency (Carpagnano et al., 2021; Weir et al., 2020), also a study determined an interaction between pneumonia and low serum levels of 25-hydroxyvitamin D [25(OH) D] (Mamani et al., 2017). The primary end-point of the current study was to identify the putative interaction between vitamin D receptor gene polymorphisms and SARS-CoV-2 infection among different variants of concern (VoC).

In our study, we observed significant differences in genotype frequencies of *FokI* and *TaqI* variants between SARS-CoV-2 infected patients and the control group ($p < 0.005$). Furthermore, the risk alleles, *FokI* T allele and *TaqI* C, were found to be statistically significant in COVID-19 patients. Also, when we grouped the studied SNPs according to genotypes (*Group1: FokI TT (Wild Type) + TaqI TT (Wild Type), Group2: FokI TC + CC (Heterozygote + Homozygote) + TaqI TC + CC (Heterozygote + Homozygote*) and analyzed in our study population, the results showed that patients with *FokI* and *TaqI* polymorphisms might tend to be more susceptible to getting infected with the SARS-CoV-2. A previous study showed that the *VDR* gene *Fok1* TT genotype and/or T allele was associated with a greater risk of infection with enveloped viruses such as Respiratory Syncytial Virus (Laplana et al., 2018). Moreover, a study in COVID-19 patients in the Cuban population showed evidence of an association between SARS-CoV-2 infection and the *VDR* gene *TaqI* polymorphism (Peralta et al., 2021), which also supported the findings of our study. A meta-analysis study indicated that vitamin D supplementation minimizes the chances of acute respiratory infections (Martineau et al., 2019). Another small-size study included 76 Spanish patients that were hospitalized due to COVID-19 disease and revealed that high doses of vitamin D application decreased the risk of intensive care unit admission (Entrenas Castillo et al., 2020). Several studies demonstrated that taking vitamin D supplements could lower the possibility of viral infection severity as well as death (Martineau et al., 2019). Freitas et al. implied that 76% of patients who died due to the COVID-19 were vitamin D deficient, whereas 59% of subjects had moderate symptoms and 64% of them had severe symptoms

(Freitas et al., 2021). Another study, which included 77 older patients in France, found that taking vitamin D supplements on a regular basis for a year prior to a COVID-19 infection was related to less severe condition and a higher chance of survival than if no vitamin D was taken (Annweiler et al., 2020). These studies support the outcomes of the current study, demonstrating a strong association between vitamin D and COVID-19 disease.

However, none of the studies determined that vitamin D receptor gene polymorphisms affect COVID-19 disease outcome as well as the SARS-CoV-2 variant impact on the disease and there was no scientific investigation that evaluates the association between SARS-CoV-2 variants of concern and *VDR* gene variations.

Furthermore, we investigated the putative impact of *VDR* gene polymorphisms on SARS-CoV-2 infection with different variants. The significant differences observed in the distribution of the *FokI* polymorphism between the three variant groups were found as statistically significant ($p < 0.005$). We supposed that the *FokI* polymorphism may be related to the severity of COVID-19 in patients. Unfortunately, we could not access the patients' clinical data, which is one of the limitations of our study, and could not confirm the association between the *FokI* polymorphism and COVID-19 severity. However, findings of a recent study by Apaydin et al. implied that serum 25 (OH) D levels had no relation to COVID-19 severity or mortality. Furthermore, it was discovered that *VDR* polymorphisms are independently related to the severity of COVID-19 and patient survival (Apaydin et al., 2022). In addition, recent Israeli research based on the first two pandemic waves, which was prior to vaccine usage, discovered a risk of having severe COVID-19 in vitamin D deficient individuals (Dror et al., 2022).

This case-control study is one of the few studies evaluating the association between SARS-CoV-2 variants of concern and *VDR* gene variations. Previous research had mostly used case reports and investigated mainly association between vitamin D levels and COVID-19 disease.

Nonetheless, due to certain limitations, our findings must be interpreted with caution. Limitations of this study included the lack of vitamin D serum level information for the participants, the lack of access to the patients' clinical data, and not including another two important *VDR* gene polymorphisms *ApaI*-rs7975232 A > C and *BsmI*-rs1544410 G > A. Further clinical trials and research with a broader range of data are required to confirm the results of our study and further investigate the association between SARS-CoV-2 variants of concern and *VDR* gene variations.

Conclusion

To conclude, the COVID-19 disease is still an important public health concern worldwide. This pandemic has severely harmed healthcare systems' ability to continue providing quality health care. As healthcare systems around the world struggle to meet the rising demand for COVID-19 care, sustaining preventive and therapeutic services is critical, especially for the most vulnerable populations such as children, the elderly, people with chronic illnesses, minorities, and people with disabilities.

It is very crucial to maintain preventive measures to avoid getting infected or minimize the severity of the disease by maintaining a safe distance from others and eating a well-balanced diet to be a healthier individual with a stronger immune system.

Our results displayed important differences in *VDR* polymorphisms' genotype distribution among different SARS-CoV-2 variants. The findings also implied that the patients with *FokI* and *TaqI* gene polymorphisms might be more susceptible to getting infected with SARS-CoV-2 variants of concern and provided evidence regarding vitamin D supplements recommendation in individuals with vitamin D deficiency/insufficiency in the peri- or post-COVID-19 pandemic.

Declarations

Ethical approval

All procedures performed in this study were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards (Approval number: YDU/202299-1451).

Conflict of Interest

The authors do not have any conflict of interest to declare.

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Data Availability Statement

The data is available upon request.

Author Contribution

Conceived and designed the analysis: G.A, MCE; Collected the data: B.M., G.A., G.T., E.M., E.U.E., H.E., H.K.S., M.C.E; Contributed data or analysis tools: B.M., G.A., G.T., E.M., E.U.E., H.E., H.K.S., M.C.E; Performed the analysis: B.M., G.A., G.T., E.M.; Wrote the paper: B.M., G.A.; revised the paper: B.M., G.A., G.T., E.M., E.U.E., H.E., H.K.S., T.S., M.C.E; supervised the project: M.C.E

References

1. Abdollahzadeh, R., Shushizadeh, M. H., Barazandehrokh, M., Choopani, S., Azarnezhad, A., Paknahad, S., Pirhoushiaran, M., Makani, S. Z., Yeganeh, R. Z., Al-Kateb, A., & Heidarzadehpilehrood, R. (2021). Association of Vitamin D receptor gene polymorphisms and clinical/severe outcomes of COVID-19 patients. *Infection, genetics,* and evolution: journal of molecular epidemiology and evolutionary

genetics in infectious diseases, 96, 105098. Advance online publication. https://doi.org/10.1016/j.meegid.2021.105098

2. Al-Anouti, F.; Mousa, M.; Karras, S.N.; Grant, W.B.; Alhalwachi, Z.; Abdel-Wareth, L.; Uddin, M.; Alkaabi, N.; Tay, G.K.; Mahboub, B.; AlSafar, H. Associations between Genetic Variants in the Vitamin D Metabolism Pathway and Severity of COVID-19 among UAE Residents. *Nutrients***2021**, *13*, 3680. https://doi.org/10.3390/nu13113680
3. 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. (2022). Effects of vitamin D receptor gene polymorphisms on the prognosis of COVID-19. *Clinical endocrinology*, *96*(6), 819–830. https://doi.org/10.1111/cen.14664
4. Annweiler G, Corvaisier M, Gautier J, Dubée V, Legrand E, Sacco G, Annweiler C on behalf of the GERIA-COVID study group. Vitamin D Supplementation Associated to Better Survival in Hospitalized Frail Elderly COVID-19 Patients: The GERIA-COVID Quasi-Experimental Study. *Nutrients*. 2020; 12(11):3377. https://doi.org/10.3390/nu12113377
5. Aranow C. Vitamin D and the immune system. J Investig Med. 2011;59(6):881-886.
6. Carpagnano, G. E., Di Lecce, V., Quaranta, V. N., Zito, A., Buonamico, E., Capozza, E., Palumbo, A., Di Gioia, G., Valerio, V. N., & Resta, O. (2021). Vitamin D deficiency as a predictor of poor prognosis in patients with acute respiratory failure due to COVID-19. *Journal of endocrinological investigation*, *44*(4), 765–771. https://doi.org/10.1007/s40618-020-01370-x
7. Cevik M, Kuppalli K, Kindrachuk J, Peiris M. Virology, transmission, and pathogenesis of SARS-CoV-2 BMJ 2020; 371:m3862 doi:10.1136/BMJ.m3862
8. Charoenngam N, Holick MF. Immunologic effects of vitamin D on human health and disease. Nutrients. 2020;12:2097. https://doi.org/10.3390/nu12072097.
9. Dror AA, Morozov N, Daoud A, Namir Y, Yakir O, et al. (2022) Pre-infection 25-hydroxyvitamin D3 levels and association with severity of COVID-19 illness. PLOS ONE 17(2): e0263069. https://doi.org/10.1371/journal.pone.0263069
10. Entrenas Castillo, M., Entrenas Costa, L. M., Vaquero Barrios, J. M., Alcalá Díaz, J. F., López Miranda, J., Bouillon, R., & Quesada Gomez, J. M. (2020). "Effect of calcifediol treatment and best available therapy versus best available therapy on intensive care unit admission and mortality among patients hospitalized for COVID-19: A pilot randomized clinical study". The Journal of steroid biochemistry and molecular biology, 203, 105751. https://doi.org/10.1016/j.jsbmb.2020.105751
11. Freitas, A.T., Calhau, C., Antunes, G. et al. Vitamin D-related polymorphisms and vitamin D levels as risk biomarkers of COVID-19 disease severity. Sci Rep 11, 20837 (2021). https://doi.org/10.1038/s41598-021-99952-z
12. Gallagher J (12 June 2021). "Covid: Is there a limit to how much worse variants can get?". *BBC*. Archivedfrom the original on 15 June 2021. Retrieved 12 June 2021.
13. Grant, W.B.; Al Anouti, F.; Boucher, B.J.; Dursun, E.; Gezen-Ak, D.; Jude, E.B.; Karonova, T.; Pludowski, P. A Narrative Review of the Evidence for Variations in Serum 25-Hydroxyvitamin D Concentration

Thresholds for Optimal Health. Nutrients 2022, 14, 639. https://doi.org/10.3390/nu14030639

14. Hendy, Mohamed; Kaufman, Samuel; Ponga, Mauricio (December 2021). "Molecular strategies for antibody binding and escape of SARS-CoV-2 and its mutations". *Scientific Reports*. **11**(1): 21735. doi:1038/s41598-021-01081-0
15. Holick MF. The vitamin D deficiency pandemic: approaches for diagnosis, treatment, and prevention. *Rev Endocr Metab Disord*. 2017;18(2):153–65.
16. Ji, Y. L., Wu, Y., Qiu, Z., Ming, H., Zhang, Y., Zhang, A. N., Leng, Y., & Xia, Z. Y. (2021). The Pathogenesis and Treatment of COVID-19: A System Review. *Biomedical and environmental sciences: BES*, *34*(1), 50–60. https://doi.org/10.3967/bes2021.007
17. Kandemiş, E., Tuncel, G., Fahrioğlu, U., Temel, Ş. G., Mocan, G., & Ergören, M. Ç. (2021). Natural selection at work? Vitamin D deficiency rates and rising health problems in young Turkish Cypriot professionals. Central European Journal of public health, 29(2), 130–133. https://doi.org/10.21101/cejph.a6117
18. Krawiec, M., & Dominiak, M. (2018). The role of vitamin D in the human body with a special emphasis on dental issues: A literature review. Dental and medical problems, 55(4), 419–424. https://doi.org/10.17219/dmp/99051
19. Laplana M, Royo JL, Fibla J (2018) Vitamin D Receptor polymorphisms and risk of enveloped virus infection: a meta-analysis. Gene 678:384–394.
20. Lee PI, Hsueh PR. Emerging threats from zoonotic coronaviruses-from SARS and MERS to 2019-nCoV. J Microbiol Immunol Infect 2020;53:365–7.
21. Lee, Y.H.; Bae, S.-C.; Choi, S.J.; Ji, J.D.; Song, G.G. Associations between vitamin D receptor polymorphisms and susceptibility to rheumatoid arthritis and systemic lupus erythematosus: A meta-analysis. *Biol. Rep.***2011**, *38*, 3643–3651. [**Google Scholar**] [**CrossRef**]
22. Li, L.; Wu, B.; Liu, J.-Y.; Yang, L.-B. Vitamin D receptor gene polymorphisms and type 2 diabetes: A meta-analysis. *Med. Res.***2013**, *44*, 235–241. [**Google Scholar**] [**CrossRef**] [**PubMed**]
23. Liu, R. et al. Positive rate of RT–PCR detection of SARS-CoV-2 infection in 4880 cases from one hospital in Wuhan, China, from Jan to Feb 2020. *Chim. Acta***505**, 172–175 (2020).
24. Mamani M, Muceli N, Basir HRG, Vasheghani M, Poorolajal J. Association between serum concentration of 25-hydroxyvitamin D and community-acquired pneumonia: a case-control study. *Int J Gen Med*. 2017; 10:423.
25. Martineau AR, Jolliffe DA, Greenberg L, Aloia JF, Bergman P, Dubnov-Raz G, Esposito S, Ganmaa D, Ginde AA, Goodall EC. Vitamin D supplementation to prevent acute respiratory infections: individual participant data meta-analysis. Health Technol Assess. 2019;23(2):1-44.
26. Martineau, A. R., Jolliffe, D. A., Hooper, R. L., Greenberg, L., Aloia, J. F., Bergman, P., Dubnov-Raz, G., Esposito, S., Ganmaa, D., Ginde, A. A., Goodall, E. C., Grant, C. C., Griffiths, C. J., Janssens, W., Laaksi, I., Manaseki-Holland, S., Mauger, D., Murdoch, D. R., Neale, R., Rees, J. R., … Camargo, C. A., Jr (2017). Vitamin D supplementation to prevent acute respiratory tract infections: systematic review and meta-

analysis of individual participant data. *BMJ (Clinical research ed.)*, *356*, i6583. https://doi.org/10.1136/bmj.i6583

27. Peralta, E. M., Rosales, Y. Z., Mesa, T. C., González, E., Pérez, Y. H., de los Ángeles González Torres, M., Balbuena, H. R., & Teruel, B. M. (2021). TaqI polymorphism of the VDR gene: aspects related to the clinical behavior of COVID-19 in Cuban patients. *The Egyptian Journal of Medical Human Genetics*, *22*(1), 83. https://doi.org/10.1186/s43042-021-00206-4
28. Raman, R., Patel, K. J., & Ranjan, K. (2021). COVID-19: Unmasking Emerging SARS-CoV-2 Variants, Vaccines and Therapeutic Strategies. *Biomolecules*, *11*(7), 993. https://doi.org/10.3390/biom11070993
29. Raoult D, Zumla A, Locatelli F, et al. Coronavirus infections: epidemiological, clinical and immunological features and hypotheses. Cell Stress. 2020;4(4):66-75.
30. Rauf, A., Abu-Izneid, T., Olatunde, A., Ahmed Khalil, A., Alhumaydhi, F. A., Tufail, T., Shariati, M. A., Rebezov, M., Almarhoon, Z. M., Mabkhot, Y. N., Alsayari, A., & Rengasamy, K. (2020). COVID-19 Pandemic: Epidemiology, Etiology, Conventional and Non-Conventional Therapies. *International journal of environmental research and public health*, *17*(21), 8155. https://doi.org/10.3390/ijerph17218155
31. Safari, I., Elahi, E. Evolution of the SARS-CoV-2 genome and emergence of variants of concern. *Arch Virol***167**, 293–305 (2022). https://doi.org/10.1007/s00705-021-05295-5
32. Shahhosseini, Nariman; Babuadze, George (Giorgi); Wong, Gary; Kobinger, Gary P. (May 2021). "Mutation Signatures and In Silico Docking of Novel SARS-CoV-2 Variants of Concern". Microorganisms. 9 (5): 926. doi:10.3390/microorganisms9050926
33. Tuncel, G., Temel, S. G., & Ergoren, M. C. (2019). Strong association between VDR FokI (rs2228570) gene variant and serum vitamin D levels in Turkish Cypriots. *Molecular biology reports*, *46*(3), 3349–3355. https://doi.org/10.1007/s11033-019-04796-6
34. Wang, B., Li, R., Lu, Z. & Huang, Y. Does comorbidity increase the risk of patients with COVID-19: evidence from meta-analysis. *Aging***12**, 6049–6057 (2020).
35. Wang, H., Chen, W., Li, D., Yin, X., Zhang, X., Olsen, N., & Zheng, S. G. (2017). Vitamin D and Chronic Diseases. Aging and disease, 8(3), 346–353. https://doi.org/10.14336/AD.2016.1021
36. Weir, E. K., Thenappan, T., Bhargava, M., & Chen, Y. (2020). Does vitamin D deficiency increase the severity of COVID-19?. *Clinical medicine (London, England)*, *20*(4), e107–e108. https://doi.org/10.7861/clinmed.2020-0301
37. WHO Coronavirus (COVID-19) Dashboard, 2022 https://covid19.who.int/
38. Yi, Y., Lagniton, P., Ye, S., Li, E., & Xu, R. H. (2020). COVID-19: what has been learned and to be learned about the novel coronavirus disease. *International journal of biological sciences,* 16(10), 1753–1766. https://doi.org/10.7150/ijbs.45134

Tables

Tables 1-2 are available in the Supplementary Files section.

Figures

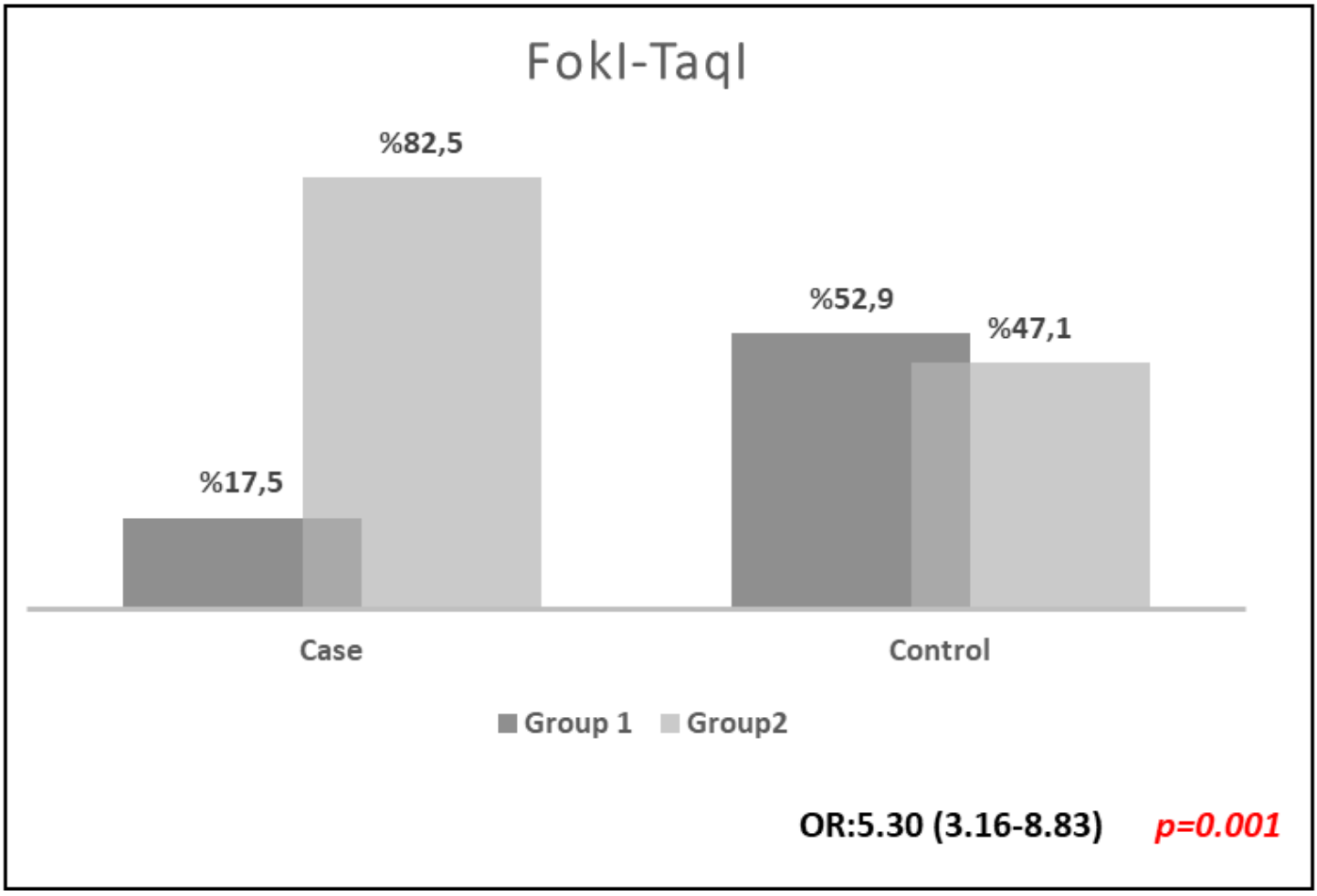

Figure 1

Comparison of *FokI* (rs10735810) and *TaqI* (rs731236) polymorphisms genotypes distributions among the study group. Group1: *FokI* TT (Wild Type) + *TaqI* TT (Wild Type), Group2: *FokI* TC (Heterozygote+ Homozygote) + *TaqI* TC (Heterozygote+ Homozygote).

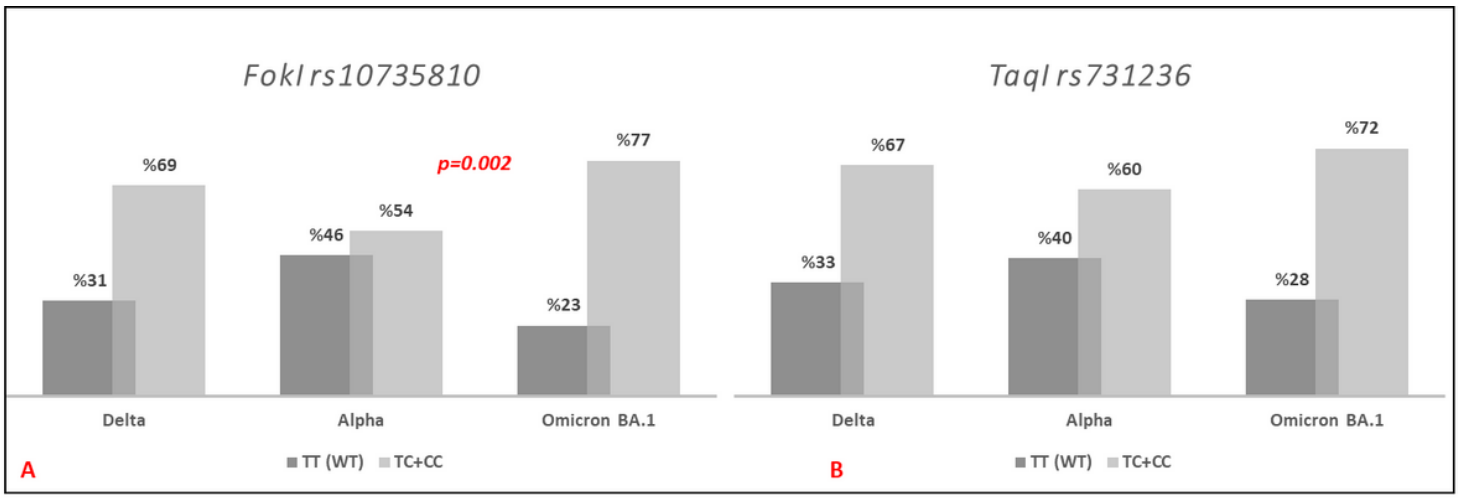

Figure 2

Genotype distributions among the SARS-CoV-2 Variants. A: *FokI* TT (Wild Type), *FokI* TC+CC (Heterozygote+Homozygote). B: TaqI TT (Wild Type), TaqI TC+CC (Heterozygote+Homozygote).

Supplementary Files

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