



Evaluation of Vitamin D Serum Levels in Pregnant Women with COVID-19 Compared with the Control Group in Pregnant Women: A Case–Control Study

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Abstract

The findings of studies on serum 25-hydroxy-vitamin D [25(OH) D] levels in pregnant women with or without coronavirus disease 2019 (COVID-19) were found to be controversial and inadequate. The present study was thus carried out at to fill the gap felt in this regard. In this case–control study, 63 pregnant women with singleton pregnancy who were infected by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), and 62 pregnant women who were matched for gestational age and not infected by COVID-19 were examined. Based on clinical symptoms, the patients with COVID-19 were divided into three groups: mild, moderate, and severe. ELISA method was adopted to measure [25(OH) D] level. The [25(OH) D] means of 23.4 ± 9.2 ng/ml and 31.2 ± 0.15 ng/ml were noted in the case and control groups, respectively ($p < 0.001$). The [25(OH) D] level of lower than 30 ng/ml was observed in 43.5% of the control group ($n = 27$) and 71.4% of the case group ($n = 45$; $p = 0.002$). Multivariate linear regression analysis to match age, gestational age, [25(OH) D] supplement use, and number of pregnancies showed that [25(OH) D] mean in the case group is 8.2 units lower, compared to the control group ($p < 0.001$). The [25(OH) D] level in pregnant women with COVID-19 is lower, compared to non-infected pregnant women. However, there is no significant relationship between [25(OH) D] level and disease severity. A sufficient level of [25(OH) D] may protect pregnant women against COVID-19.

Keywords Coronavirus disease 2019 · 25-hydroxy-vitamin D · Pregnancy

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Introduction

Being made in skin through sunlight, vitamin D is considered as unique [1]. In addition to bone and calcium metabolism, vitamin D plays an important role in regulating the body's immunity by inducing phagocytes and modulating the effects of Th1 and Th2 and regulatory T cells [2]. Vitamin

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D deficiency in pregnancy is common [3]. Adequate levels of [25(OH) D] during pregnancy are necessary for a proper maternal response to calcium needs of fetus and calcium use by baby. 25-hydroxy-vitamin D [25(OH) D] deficiency during pregnancy causes potentially harmful consequences for mother and fetus. Some studies have shown that [25(OH) D] deficiency during pregnancy is associated with an increase in preeclampsia, prematurity, and low birth weight risk [4].

Coronaviruses are one of the most important causes of diseases in humans and animals; and an unprecedented global effort is underway to prevent its spread. In fact, coronaviruses have become one of the main threats to human health in the last two decades [5]. In the absence of a safe and effective vaccine, and the absence of specific drug treatment, the only solution to prevent its spread is public education, and proper prevention and control [6]. Some studies have indicated that the decrease in or deficiency of [25(OH) D] is associated with an increase in the risk of contracting Coronavirus Disease 2019 (COVID-19) [7, 8]. Some studies have also found that lack of vitamin D is associated with an increase in hospitalization risk, more severe disease, or even death [9]. Pregnant women have lower averages of [25(OH) D], compared to non-pregnant ones [10, 11]. However, some studies reported that no significant effect was observed in such patients by administering [25(OH) D] [12]. Yet, no study has been conducted on the serum level of [25(OH) D] in Iranian pregnant women, and its relationship with the disease severity, this study thus seeks to investigate the serum level of [25(OH) D] in pregnant women with COVID-19.

Materials and Methods

This case–control study was conducted following approval by the Hamedan University Ethical Committee (Code: IR.UMSHA.REC.1401.174) at the Fatemieh Hospital in Hamedan between May and September 2022. The sample size was calculated based on the findings of a study done by Sinaci et al. [13] with a power of 90% and a Type 1 Error of 0.05 by using G*Power software. Inclusion criteria in the case group included singleton pregnancy and positive COVID-19 test, while the control group had no signs or symptoms of COVID-19. In both groups, multiple pregnancies, systemic disease such as diabetes, cardiovascular disease, and blood pressure caused by pregnancy, asthma, thrombolytic diseases, inflammatory bowel disease, and other infectious diseases were among the exclusion criteria.

In this study, pregnant women with singleton pregnancy and COVID-19 infection in the case group were confirmed by an internist based on the results of lung test, PCR, and CT, whereas the pregnant women in the control group who had singleton pregnancy, were the same as the case group in terms of gestational age, and were referred to the Fatemieh Hospital for pregnancy care, did not have any symptoms of COVID-19.

In this study, the severity of COVID-19 disease was done based on the guidelines of the World Health Organization. Patients with mild to moderate disease, who were classified as symptomatic patients, did not need oxygen therapy, but patients with severe disease received oxygen support [14].

Demographic data (e.g., age) and obstetric data (e.g., gestational age, number of pregnancies, and number of children) were collected from both groups. To determine the serum level of [25(OH) D], which is the active form of the vitamin, samples were taken from all study mothers in the laboratory of the Fatemieh Hospital, and its level was measured by ELISA. The serum level of [25(OH) D] ≥ 30 ng/ml was considered as the sufficient level of [25(OH) D].

Statistical Analysis

The data were analyzed by SPSS v20 at the statistically significant level 0.05. The data were described by mean and standard deviation for quantitative variables, and ratio and percentage for qualitative variables. Chi-square test or Fisher's Exact Test was run to compare qualitative variables, and t-test or Mann–Whitney was conducted for quantitative variables.

Before the data analysis, the aforementioned potential confounding factors were defined by reviewing the literature, and the effect of each one on the [25(OH) D] level was assessed. The variables were then selected with p values < 0.3 to include in the multiple regression analysis. After fitting the multiple regression model, the variables with p values > 0.3 , the elimination of which led to better fitting, were removed from the model (backward stepwise). Finally, the multiple linear regression analysis was conducted to examine the effect of COVID-19 disease on the serum levels of [25(OH) D] by adjusting the effect of confounding variables.

Results

There were no statistical differences between the two study groups with respect to age, gestational age, pregnancy, and vitamin D supplementation ($p > 0.05$). Nine patients in the case group (14.5%) were suffering from an underlying disease, and the mean hospitalization period of the case group was 10.3 ± 4.2 days (Table 1). The serum concentration of [25(OH) D] was 31.2 ± 15.0 ng/ml in the control group, and 23.4 ± 9.2 ng/ml in the case group, and the results of independent t -test showed a statistically significant difference between the two groups in this regard ($p = 0.001$). Taking both groups into account, 72 people (57.6%) had insufficient or low, and 53 people (42.4%) had sufficient [25(OH) D] serum levels (≥ 30 ng/ml). Also, 27 people in the control group (43.5%) and 45 people in the case group (71.4%)

Table 1 Comparison of baseline variables within two study groups

Variable	Control group, <i>n</i> = 63	Case group, <i>n</i> = 62	<i>P</i>
Age (yr.), mean \pm SD	28.8 \pm 8.3	29.5 \pm 8.4	0.611*
Gestational age (week), mean \pm SD	26.9 \pm 7.2	29.1 \pm 6.7	0.068*
Gravid, mean \pm SD	2.5 \pm 0.9	2.7 \pm 0.7	0.441**
Parity, mean \pm SD	1.4 \pm .07	1.6 \pm 1.1	0.387**
Hospitalization,(days), mean \pm SD	-	10. 3 \pm 4.2	-
Vitamin D supplement, <i>n</i> (%)	47 (75.8)	44 (69.8)	0.454 †
Underline disease, <i>n</i> (%)	-	9 (14.5)-	

*: *t*-test, **: Mann–Whitney, †: chi2

had insufficient levels of [25(OH) D], and such a difference was found to be statistically significant. Of 63 patients with COVID-19, 47 patients (74.6%) had mild or moderate disease severity and 16 patients (25.4%) had a severe or critical disease.

As can be seen, assuming other variables as constant, as a person changes from the control group to the case group, the mean level of [25(OH) D] decreases significantly by 8.2 units. Assuming other variables as constant, the mean level of [25(OH) D] decreases significantly by 0.284 units for one year of age increase. There was no significant relationship between gestational age and supplement use with [25(OH) D] level (Table 2).

There was no significant relationship between disease severity and age, obstetric variables, and mean [25(OH) D] level. The [25(OH) D] level in patients with severe or critical diseases was lower, compared to patients with mild or moderate disease severity, but there was no statistically significant difference between mild and moderate patients (Table 3).

Discussion

The findings showed that the serum level of [25(OH) D] in pregnant women with COVID-19 who were adjusted in terms of possible confounding variables was 8.2 units lower, compared to non-infected people. On the other hand, 71.4% of the people in the case group compared to 43.5%

of the people in the control group had insufficient levels of [25(OH) D] ($p=0.002$). However, the level of [25(OH) D] did not show any significant relationship with the disease severity. As far as the researchers are concerned, and based on the extensive review of the related literature, this is the first study conducted on the serum level of [25(OH) D] in Iranian pregnant women with COVID-19.

No approved treatment method has yet been found for COVID-19. Therefore, most of the measures aimed at preventing, improving the immune system, and reducing complications associated with it [15]. Studies have shown that people without an optimal level of [25(OH) D] are more vulnerable to infectious diseases, like COVID-19 [16, 17]; however, the results of studies on the amount of [25(OH) D] serum level in pregnant women and its effect on the disease severity have been controversial.

In a case–control study conducted by Sinaci et al. [13] in Turkey, 159 women with singleton pregnancy and positive COVID-19 tests, and 332 pregnant women without COVID-19 were compared. The results showed that the serum level of [25(OH) D] in women with COVID-19 is statistically significantly lower (12.46 \pm 6.46 ng/mL vs. 137 \pm 18.76 ng/mL, $p=0.004$).

Contrary to the findings of the present study, in another case–control study by Tekin et al. [18], 147 pregnant women with COVID-19 and 300 age-matched cases were compared in terms of gestational age. The findings indicated that the serum concentration of [25(OH) D], at the beginning of admission, was 36.6 \pm 26.8 nmol/L in pregnant women

Table 2 Regression model of variables of age, gestational age, supplement use, and control or case group 25(OH) D dependent variable

Variable	Unstandardized Coefficients		Standardized Coefficients	<i>P</i>
	Coefficient	SE	Beta	
(Constant)	44.87	6.405		<0.001
Group	-8.200	2.244	-.317	<0.001
Age	-.284	.132	-.183	.034
Gestational age	.198	.145	.119	.173
Vitamin D supplement	-1.872	2.486	-.064	.453

Table 3 Relationship between the disease severity and the examined variables

Variable	Mild to moderate mean \pm SD	Severe mean \pm SD	<i>P</i>
Age (yr.)	29.5 \pm 8.1	29.6 \pm 9.8	0.983*
Gestational age (week)	28.9 \pm 7.5	28.6 \pm 6.1	0.873*
Gravid	2.1 \pm 1.1	1.8 \pm 1.3	0.216**
Parity	0.9 \pm 1.1	0.7 \pm 1.1	0.275**
25-hydroxy-vitamin D	23.8 \pm 9.6	22.0 \pm 8.3	0.498

*: *t*-test, **: Mann–Whitney,

infected with SARS-CoV-2, and 31.3 ± 20.7 nmol/L in healthy women, which was also found to be statistically significant; however, when they classified the level of [25(OH) D], it was found that 75.5% of infected people and 82.3% of non-infected people have [25(OH) D] deficiency, and no significant difference was noted between the two groups ($p=0.090$). In studies conducted on non-pregnant women, no correlation between [25(OH) D] levels and COVID-19 has been reported [15, 19]. Based on the findings of a study done by Meltzer et al. [17], which was a large-scale study, serum levels of [25(OH) D] even one year before the COVID-19 pandemic increased the risk of Covid disease.

According to the findings of the study, although the [25(OH) D] level in patients with severe or critical diseases was lower than in those with mild diseases, no statistically significant difference between these two groups was observed, which might be due to the small number of patients examined. However, in connection with the serum level of [25(OH) D] and the disease severity in pregnant patients, the results of the studies are controversial. In a study carried out by Tekin et al. [18] in 2021, it was also found that in pregnant patients with COVID-19, the [25(OH) D] has no significant relationship with the disease severity (RR=0.568, 95% CI [0.311–1.036]; $p=0.065$). However, in a study conducted by Seven et al. [20] in 2021, the levels of [25(OH) D] in pregnant patients with COVID-19, asymptomatic or mild, were statistically significantly lower (15.5 ± 10.0 vs. 13.0 ± 0.12), compared to severe disease and with a poor prognosis. In a study conducted in Turkey, the people with mild disease severity had higher levels of [25(OH) D], compared to those with moderate or severe severity (13.69 vs. 9.06) [13]. In a meta-analysis study carried out by Crafa et al. [21], it was concluded that people with lower serum levels are at a higher risk for the disease with greater severity and the risk of death. However, in a meta-analysis study done by Amrein et al. [22], the findings did not indicate any significant relationship between the level of [25(OH) D] in COVID-19 patients with the rate of hospitalization in ICU, invasive and non-invasive mechanical ventilation, and mortality. According to the findings of Hernandez et al.'s study [23], even though the level of [25(OH) D] was lower in patients with COVID-19 compared to healthy people, it did not have any significant relationship with the disease severity.

The results of studies show that pregnant women without sufficient levels of [25(OH) D] are exposed to several risks, including preeclampsia or blood pressure caused by pregnancy, prematurity, gestational diabetes, small for gestational age, increase in mortality, and perinatal and maternal complications. Therefore, vitamin D supplementation may prevent the risk of preeclampsia or high blood pressure in pregnant women with COVID-19 [24]. Considering the relatively simple measurement of the serum level of the vitamin and the low cost of vitamin D supplements, it is considered

as one of the useful ways for this disease and other viral diseases and reduction of pregnancy complications due to the relatively high prevalence of [25(OH) D] deficiency in pregnant women. Liu et al. in 2020 stated that a single dose of 300,000 international units of vitamin D may play a role in the prevention and treatment of COVID-19 [25].

COVID-19 increases inflammatory cytokines such as interleukin-1 β , tumor necrosis factor- α , interleukin 8, interleukin 12, and especially interleukin 6 [26]. In addition to its role in the skeletal system and calcium metabolism, vitamin D plays an important role in the immune system [27]. The possible mechanisms for the role of vitamin D in viral diseases including COVID-19 may be due to its direct anti-inflammatory activity in the lung due to its local inhibitory effect on nuclear factor KB and the reduction of inflammatory cytokines due to the activity of activated protein kinase (protein kinase). The change of monocytes to macrophages at the tissue level and the increase in anti-inflammatory cytokines are considered as other reasons why vitamin D can be effective in infectious diseases [22].

Conclusion

According to the findings of the present study, the [25(OH) D] level in pregnant women with COVID-19 is lower, compared to non-infected pregnant women. However, there is no significant relationship between [25(OH) D] level and disease severity. A sufficient level of [25(OH) D] may protect pregnant women against COVID-19.

Authors' Contribution SK&MS: reviewed and edited the manuscript, AG&RA: conceptualized and designed the study, ETG: collaborated on the analysis, RB: critically reviewed the manuscript, and NR: conceptualized and designed the study. All authors reviewed the manuscript and they agree with the final version.

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Code Availability Not applicable.

Declarations

Ethics Approval Hamadan University of Medical Sciences (IR. UMSHA.REC.1401.174).

Consent to Participate Consent to participate from all patients was obtained.

Consent for Publication Not applicable.

Conflicts of Interest The authors declare no competing interests.

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