Comparative Study between Serum Vitamin D Levels in Patients with Pre-eclampsia versus Healthy Pregnant Women

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Comparative Study Between Serum Vitamin D Levels in Patients With Preeclampsia Versus Healthy Pregnant Women

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Abstract

**Background:** Preeclampsia etiology remains a mystery; a multitude of theories propose aberrant placental implantation and deviant trophoblastic invasion. Clinical studies examining the link between vitamin D concentrations and poor pregnancy results like preeclampsia have shown mixed findings.

**Objective:** To measure serum concentrations of vitamin D in preeclamptic gestating women and to compare them with their healthy counterparts.

**Patients and methods:** After the 20th week of gestation, a prospective, case–control research was conducted on 100 gestating women: 50 gestating women with preeclampsia and 50 gestating women without preeclampsia (controls). Patients were selected to assess the total 25-OH vitamin D using an enzyme immunoassay technique.

**Results:** Our findings showed that there was statistically substantial reduction in median vitamin D serum concentrations of preeclampsia patients (17.47 ± 4.86 mg/dl) compared with controls (27.15 ± 5.94 mg/dl) \( (t = -8.909, P < 0.001) \). It also exhibited a negative relationship with systolic and diastolic blood pressure, as well as white blood cell \( (P < 0.001) \). In addition, the receiver operating characteristic curve analysis showed that the optimum cutoff for vitamin D was 24.05 mg/dl for distinguishing pregnant women with preeclampsia from normotensive pregnant women with a sensitivity of 88% and specificity of 88% with an area under the receiver operating characteristic curve of 0.909 (95% confidence interval: 0.845–0.974).

**Conclusion:** When preeclampsia pregnant women were analyzed to normotensive pregnant women, plasma vitamin D concentrations were considerably lower. As a result, this research has suggested the potential of a link between maternal vitamin D insufficiency and the incidence of preeclampsia.

**Keywords:** Preeclampsia, Vitamin D deficiency, Vitamin D

1. Introduction

Preeclampsia, affecting between 2 and 8% of pregnancies, is one of the world's prominent triggers of perinatal morbidity and mortality.¹

Though the etiology of preeclampsia remains a mystery, a heightened understanding of the preeclampsia pathogenesis has raised the possibility of decreased spiral artery remodeling at the trophoblastic invasion time instigating placental insufficiency followed by the release of inflammatory markers into the maternal systemic circulation with subsequent endothelial dysfunction, which is the characteristic pathological preeclampsia finding.²

Vitamin D deficiency (VDD) is nowadays a prevalent epidemic. Maternal VDD is a widespread public problem. Pregnant women all around the globe suffer from high rates of VDD.³

Clinical reports linking vitamin D levels to unfavorable pregnancy consequences comprising preeclampsia, gestational diabetes, preterm and
cesarean delivery, and low birth weight have shown contradictory results.4

Vitamin D may have an impact on implant and placental development during pregnancy, potentially due to its angiogenic, anti-inflammatory, and immune-modulatory properties. VDD in the mother may impair the health of both the mom and the baby by elevating the generation of inflammatory cytokines and promoting the activation of T-regulating cells.5

The function of maternal vitamin D levels in the establishment of preeclampsia is gaining popularity. Therefore, the goal of this research was to measure vitamin D levels in gestating women preeclampsia and to compare them with their healthy counterparts.

2. Patients and methods

After the 20th week of pregnancy, a prospective case–control research was done on 100 gestating women: 50 gestating women with preeclampsia and 50 gestating women without preeclampsia (controls). During the study period, which lasted from the beginning of March 2021 to the end of December 2021, participants were recruited from the Al-Hussein Hospital of Al-Azhar University’s Obstetric clinics. The ethics committee was approved by the quality education assurance unit of Al-Azhar University Egypt’s Faculty of Medicine.

Inclusion criteria were: maternal age between 18 and 40 years, gestational age after the 20th week of pregnancy, while exclusion criteria were maternal systemic disorder (high blood pressure, diabetes, chronic renal sickness, and ischemic heart disorder), history of immunosuppression intake, and history of previous poor pregnancy outcomes, history of smoking, and antenatal vitamin or mineral supplementation.

The chosen patients underwent a comprehensive history taking as well as full general and abdominal assessment, and ultrasound evaluation at the Ultrasound Unit of Al-Hussein Hospital of Al-Azhar University. The ultrasound equipment used was (Mindray DC-N2, Dakahlia, Mansoura city, Egypt) with a 3.5–5-MHz transabdominal probe and a 5–9 MHz transvaginal probe. The investigation included: complete blood count, blood grouping and Rh, liver and kidney functions, coagulation profile, fasting and postprandial blood sugar, and urinary protein levels.

Determination of 25-OH vitamin D: from each participant a venous blood sample of around 5 ml was taken. After that, a clear serum sample was produced by centrifugation at ambient temperature for 10 min at 3500 rpm, followed by storage at −70 °C for no more than 1 month until analysis. The total 25-OH vitamin D was determined according to the enzyme immunoassay method using a Tests Kits obtained from (Calbiotech Inc., Catalog No: VD220B., 1935 Cordell CT, El Cajon, CA 92020 USA).

2.1. Statistical analysis

SPSS (North American headquarters, 2000 Centregreen Way, Suite 300, Cary, North Carolina 27513, USA), version 23 was used for statistical analysis of data. The Shapiro–Wilks test was performed to determine if the variables had a normal distribution. The mean, SD, or median and range were used to convey numerical data. Percentages were used to summarize categorical data. A two-tailed Student’s t-test was used to examine the significance of the variation between groups. The $\chi^2$ test was also used to examine qualitative factors. $P$ values of less than or equal to 0.05 were deemed statistically substantial.

3. Results

After the 20th week of gestation, the researchers recruited 100 gestating women: 50 with preeclampsia and 50 normotensive gestating women (controls). The preeclampsia group had an average age of 28.3 ± 3.81 years, whereas the control group had an average age of 26.78 ± 3.17 years. The average age of cases in the preeclampsia group and control participants differed statistically significantly ($P = 0.033$). Furthermore, these findings revealed that there was no statistically substantial variation in body mass index ($P = 0.644$), gravidity ($P = 0.073$), or parity ($P = 0.459$) between the two groups tested. The median systolic blood pressure in patients was shown to be $159.4 ± 21.79$ mmHg, compared with $113.6 ± 10.05$ mmHg among controls, indicating that a rise in both systolic and diastolic blood pressure was related to the incidence of preeclampsia ($P < 0.001$). Furthermore, the median diastolic blood pressure in preeclampsia patients was $103.9 ± 12.38$ mmHg, compared with $71 ± 6.47$ mmHg in controls. The average gestational age at birth was found to be substantially lower in preeclampsia patients ($36.12 ± 1.49$) compared with control cases ($38.08 ± 1.29$) ($P < 0.001$). In the preeclampsia group, 23 (46%) of the patients had proteinuria levels ranging from 1 to 2 to 3–4, whereas 27 (54%) of the cases had a level ranging from 3 to 4. There was substantial variation between the two groups when it came to the presence of protein in urea, which was higher in preeclampsia patients ($P < 0.001$). When it came to neonatal birth weight, 11 (22%) neonates delivered to women with preeclampsia had weights ranging from 1500 to 2499 g, while the other 39 (78%) babies had
weights more than or equal to 2500 g. The median birth weight in the preeclampsia group was 2624 ± 344.59 g, compared with 3295 ± 329.88 g in the control group (P < 0.001). The median ± SD of hematological values was 12.28 ± 1.22 versus 13.2 ± 1.22 g/dl for hemoglobin and 4.2 ± 0.635 versus 4.11 ± 0.34 × 10^6/μl for red blood cell (RBC) count, according to the findings. In addition, in the preeclampsia and control groups, the mean platelet count was 249.849 ± 82.982 versus 266.612 ± 46.973 × 10^3/μl, and the median white blood cells (WBC) count was 10.395 ± 2.963 versus 6.704 ± 1.929. When comparing patients with preeclampsia to healthy controls, the mean value of blood vitamin D was compared. The median vitamin D level exhibited a statistically substantially lower median level of blood vitamin D (17.47 ± 4.86 vs. 27.15 ± 5.94 in controls) (t = −8.909, P < 0.001) (Table 1).

Furthermore, the median vitamin D level exhibited a substantial positive connection with age, gestation age, and birth weight (P < 0.05) in the present research. It also exhibited a negative relationship with systolic and diastolic blood pressure, as well as WBC (P < 0.001) (Table 2).

### Table 1. Patients’ and controls’ demographic and biochemical parameters.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Groups</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>Preeclampsia group (N = 50)</td>
<td>Healthy controls (N = 50)</td>
</tr>
<tr>
<td>Gestational age at delivery (weeks)</td>
<td>28.3 ± 3.81</td>
<td>26.78 ± 3.17</td>
</tr>
<tr>
<td>BMI at enrollment</td>
<td>36.12 ± 1.49</td>
<td>38.08 ± 1.29</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>27.73 ± 4.54</td>
<td>28.07 ± 2.39</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>139.4 ± 21.79</td>
<td>113.6 ± 10.05</td>
</tr>
<tr>
<td>Gravidity</td>
<td>103.9 ± 12.38</td>
<td>71 ± 6.47</td>
</tr>
<tr>
<td>Parity</td>
<td>2.42 ± 1.11</td>
<td>2.88 ± 1.41</td>
</tr>
<tr>
<td>Appearance of proteinuria</td>
<td>1.06 ± 0.98</td>
<td>1.2 ± 0.9</td>
</tr>
<tr>
<td>None</td>
<td>0</td>
<td>50 (100)</td>
</tr>
<tr>
<td>1–2</td>
<td>23 (46)</td>
<td>0</td>
</tr>
<tr>
<td>3–4</td>
<td>27 (54)</td>
<td>0</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>2624 ± 344.59</td>
<td>3295 ± 329.88</td>
</tr>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>12.28 ± 1.22</td>
<td>13.2 ± 1.22</td>
</tr>
<tr>
<td>RBC (× 10^6/μl)</td>
<td>4.2 ± 0.64</td>
<td>4.11 ± 0.34</td>
</tr>
<tr>
<td>Lymphocytes (μl)</td>
<td>10.395 ± 2.963</td>
<td>6.704 ± 1.929</td>
</tr>
<tr>
<td>PLT (× 10^3/μl)</td>
<td>249.849 ± 82.982</td>
<td>266.612 ± 46.973</td>
</tr>
<tr>
<td>Vitamin D (mg/dl)</td>
<td>17.47 ± 4.86</td>
<td>27.15 ± 5.94</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SD or n (%).

*P value less than or equal to 0.05.

**P value less than or equal to 0.01.

### Table 2. Connection between vitamin D and other variables investigated.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Vitamin D</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin D</td>
<td>1.000</td>
<td>–</td>
</tr>
<tr>
<td>Age</td>
<td>0.208</td>
<td>0.037*</td>
</tr>
<tr>
<td>Gestational age</td>
<td>0.417</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>Birth weight</td>
<td>0.563</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>Gravidity</td>
<td>0.181</td>
<td>0.072</td>
</tr>
<tr>
<td>Parity</td>
<td>0.135</td>
<td>0.181</td>
</tr>
<tr>
<td>BMI</td>
<td>0.023</td>
<td>0.823</td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>−0.438</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>Diastolic blood pressure</td>
<td>−0.499</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>Proteinuria</td>
<td>−0.130</td>
<td>0.368</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>0.169</td>
<td>0.093</td>
</tr>
<tr>
<td>WBCs</td>
<td>−0.439</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>Platelet count</td>
<td>0.090</td>
<td>0.376</td>
</tr>
<tr>
<td>RBCs</td>
<td>−0.106</td>
<td>0.303</td>
</tr>
</tbody>
</table>

RBC, red blood cell; WBC, white blood cell.

*: P ≤ 0.05; **: P ≤ 0.01.

### Table 3. Diagnostic values of vitamin D in the study participants (N = 100).

<table>
<thead>
<tr>
<th>Cutoff</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin D</td>
<td>24.05</td>
<td>88%</td>
<td>88%</td>
<td>88%</td>
</tr>
</tbody>
</table>

NPV, negative predictive value; PPV, positive predictive value.

Finally, an analysis of the receiver operating characteristic (ROC) curve revealed that blood vitamin D had substantially greater diagnosing
accuracy in predicting the onset of preeclampsia. ROC curve showed that the optimum cutoff for vitamin D was 24.05 mg/dl for distinguishing pregnant women with preeclampsia from normotensive pregnant women with a sensitivity of 88% and specificity of 88%; the area under the ROC curve had a value of 0.909 (95% confidence interval: 0.845–0.974) (Table 3 and Fig. 1).

4. Discussion

Given the relevance of maternal vitamin D status in the genesis of preeclampsia, the goal of this research was to test blood vitamin D values in pre eclamptic gestating women and compare them with their healthy counterparts.

In the present research, after the 20th week of pregnancy, 100 pregnant females were included in the research: 50 with preeclampsia and 50 with normal blood pressure (controls). During the study period, from March to December 2021, they all visited Al-Hussein Hospital of Al-Azhar University’s obstetric clinics. According to our findings, there was no statistically substantial variation across the two groups regarding BMI ($P = 0.644$), gravidity ($P = 0.073$), or parity ($P = 0.459$).

And, in the current research, the median gestation age at delivery was 37.1 ± 1.7 weeks in all studied cases who completed gestation. The median gestation age at labor in patients with preeclampsia was 36.12 ± 1.49 weeks, while it was 38.08 ± 1.29 weeks in control cases. These findings demonstrated that in the preeclampsia group, the median gestation age at birth was substantially lower than in the control group ($P < 0.001$).

In line with our results, Bakacak et al.$^6$ and Gogaram and Misha$^7$ reported that the gestation period of those with preeclampsia was substantially reduced than that of the control group.

Nevertheless, in the study by Karpa et al.$^5$, the median gestation age at labor in the preeclampsia group was 37.80 ± 2.17 versus 38.06 ± 1.89 weeks in the control counterparts without statistically significant discrepancy, which was also analogous to the research accomplished by Abedi et al.$^8$ and Mohaghegh et al.$^9$

There is widespread agreement that delivering the baby and placenta as soon as possible is the only effective management for preeclampsia, which is linked to an enhanced threat of premature births and its associated complications, such as an elevated need for neonatal ICU admissions, a higher newborn death rate, and so on.$^{10}$

Regarding the mode of delivery, we found that 35 (70%) cases of preeclampsia were delivered by cesarean section (CS), while the remaining 15 (30%) were delivered through the vaginal route. A statistically significant higher occurrence of CS rates in the preeclampsia group than in the control group ($P = 0.041$) was identified.

These results were in line with the escalating rate of CS both worldwide and nationally. In much of the developed world, cesarean delivery has grown more prevalent.$^{11}$

The CS rate is much higher than the rate of vaginal birth as most healthcare records reflect a predilection for CS for pregnancy termination.$^{12}$

The CS rate was higher than that of women who had normal vaginal delivery to some extent in this research. This is in contrast to the results of Kumari et al.$^{13}$ researches, which found that the rate of vaginal birth was greater than the rate of CS.

CS, however, has consistently been shown to be greater in preeclampsia/eclampsia women in most research throughout the globe.$^{14}$

Considering the neonatal birth weight after delivery, our results found that 11 (22%) of the newborns, who were born to preeclampsia mothers had weight in the range of 1500–2499 g, while other cases ($n = 39$) had birth weight more than 2500 g. In the control group, all babies had weight at the time of birth more than 2500 g. Mean neonatal birth weight was significantly lower (2624 ± 344.6 g) in the preeclampsia group than that of the control group (3295 ± 329.88 g).

In concordance with our findings, Bakacak et al.$^6$ reported that birth weight was considerably lower in the preeclampsia patient group (2.434.1 ± 854.29 g) compared with the healthy counterparts (3.248.8 ± 548.68 g) ($P < 0.001$).

Lower birth weight may be either due to preterm delivery or concomitant intrauterine growth.

Fig. 1. Vitamin D ROC curve for distinguishing patients with preeclampsia from healthy controls. ROC, receiver operating characteristic.
IUGR is usually associated with preeclampsia, according to the literature; however, this result might be due to other illnesses that affect placental blood circulation. When preeclampsia and IUGR occur at the same time, the pregnancy outcomes are poorer than when preeclampsia and IUGR occur separately.

Furthermore, as compared with healthy controls, there was substantial drop in the median value of hemoglobin concentrations and WBC counts in preeclampsia patients ($P < 0.001$). However, there was no statistically substantial variation in platelet count and RBC count between the two groups ($P > 0.05$).

In healthy gestation, in the sixth week, plasma volume tends to increase with an unbalanced increase in the RBC mass. These result in augmented plasma transport even with very low hemoglobin count. Nevertheless, this surge in plasma is a signal of normal fetal growth.

The results of this research are reinforced by other studies as it was reported that hemoglobin levels and RBC index in the two groups had significant differences. Preeclampsia women had lower hemoglobin levels versus normal mothers ($P < 0.001$); yet, the RBC and reticulocyte numbers did not show a significant difference between both groups.

In research conducted by Nasiri et al., who examined the hemoglobin levels to predict preeclampsia, it has been reported that the mean hemoglobin value of women with and without preeclampsia in trimesters one to two and three had a difference of 0.46 g/dl and $P$ value of 0.003.

Regarding the aim of our study and the comparison of the average blood vitamin D concentrations among preeclampsia patients and control groups, our findings demonstrated that the median level of serum vitamin D was statistically substantially lower in preeclampsia patients ($17.47 \pm 4.86$ mg/dl) compared with controls ($27.15 \pm 5.94$ mg/dl) ($t = -8.909, P < 0.001$). In the studied participants, the mean vitamin D had substantial positive relation with age, gestation age, and birth weight ($P < 0.05$). Also, it had a substantial negative relationship with systolic and diastolic blood pressure and WBC ($P < 0.001$). In addition, the ROC curve analysis showed that the optimum cutoff for vitamin D was 24.05 mg/dl for distinguishing pregnant women with preeclampsia from normotensive pregnant women with a sensitivity of 88% and specificity of 88%; the area under the ROC curve had a value of 0.909 (95% confidence interval: 0.845–0.974).

Our findings support what previous research has shown, Aghajafari et al., in which preeclampsia patients had low serum 25-OH vitamin D concentrations than healthy pregnant normotensive controls.

In agreement with our results, Bakacak et al., reported that the mean serum vitamin D levels were $23.7 \pm 5.93$ and $19.3 \pm 4.31$ ng/ml in healthy controls versus women with preeclampsia, respectively. Sadin et al. conducted a study to determine the concentrations of 25-OH vitamin D in women with and without preeclampsia and discovered that levels below 10 ng/ml were linked with a 15-fold increased risk of preeclampsia as compared with healthy controls.

Singla et al. also showed that mean serum 25-OH vitamin D was statistically substantially decreased among preeclampsia cases (mean $\pm$ SD = $9.7 \pm 4.95$ ng/ml) as matched with normotensive controls (mean $\pm$ SD = $14.8 \pm 6.68$ ng/ml) ($P = 0.0001$).

These results were also in line with those of Mehmoord and Karim, who showed that the serum 25-OH vitamin D concentration was substantially reduced in hypertensive cases (preeclampsia, eclampsia, and gestational hypertension) compared with controls (6.78 ng/ml in instances and 9.43 ng/ml in controls) ($P = 0.002$).

In a study by Pulei, preeclampsia patients and normotensive controls had a mean blood vitamin D status of $20.8 \pm 10.2$ and $28.6 \pm 7.9$ ng/ml, respectively ($P < 0.001$).

Dhillon et al. also observed that a lower vitamin D status was detected in women with preeclampsia compared with healthy ones, which is consistent with the present study.

In the Sharma et al. study, blood vitamin D status was lower in women with hypertension abnormalities of pregnancy (median $= 13.66 \pm 7.358$ ng/ml) compared with normal normotensive prenatal women (mean $= 21.14 \pm 8.141$ ng/ml).

Osman et al. also in another Egyptian study found that the median 25-OH vitamin D value was reduced in the preeclampsia group ($14.8 \pm 5.4$ ng/ml) than in pregnant controls ($19.5 \pm 6.5$ ng/ml) ($P = 0.002$). Tabesh et al. in a meta-analysis of this issue established a link between reduced 25-OH vitamin D level and preeclampsia.

Likewise recently, Karpa et al. reported that the vitamin D level was $8.87 \pm 4.66$ in the preeclampsia group and $25.83 \pm 7.07$ in the control counterparts ($P < 0.0001$).

Contrary to the current research, Umar et al. discovered that the variation in vitamin D levels between the two groups of interest was not substantial.

In contrast to our results, Hashemipour et al. carried out a case–control research on 75 healthy
pregnant women and 74 preeclampsia complicated pregnancies. They found that median serum vitamin D levels were 27.7 ± 15.3, 22.9 ± 15.9, and 27.6 ± 16.6 in normotensive, moderate preeclampsia, and extreme preeclampsia groups with P value more than 0.05. Consequently they reported that 25-OH vitamin D deficiency was not altered between groups.

Another Egyptian study conducted by Mohammed et al.° reported that serum vitamin D level was reduced in the preeclampsia group than the normotensive group, but this was not statistically substantial.

Vitamin D metabolism is dramatically altered in preeclampsia compared with normal pregnancies. This might be due to decreased circulating calcitriol concentrations due to diminished placental 1-α hydroxylase activity compared with normotensive women.°

Vitamin D insufficiency was found in 26–98% of pregnant women in studies conducted in the United States, Australia, South Asia, and the Middle East. Vitamin D insufficiency was found in 66–100% of dark-skinned females.°

Although not universal, the cutoff level for vitamin D in the blood has been established at 20 ng/ml in recent research, although it was previously recognized at 15 ng/ml.°

Low vitamin D level has been linked to a variety of pathways that have been proposed to cause preeclampsia. First, vitamin D is thought to have a role in the production and regulation of genes involved in the early stages of placental growth. Second, vitamin D is assumed to have a substantial endocrine suppressor effect in renin production, which is important for the renin–angiotensin system regulation. Finally, it is thought to have anti-inflammatory effects and participates in placental immunomodulation.°

The link between vitamin D and preeclampsia is complicated; some researchers have suggested that low vitamin D concentrations in the second trimester might be a sign of preeclampsia. It is still debatable if vitamin D supplements should be used during pregnancy. The Royal College of Obstetricians and Gynecologists recommended standard vitamin D supplementation in pregnancy in 2014, mostly for at-risk instances such as dark skin, cases concealed from the sun, and obese patients.°

On constructing the ROC curve model a systemic review conducted by Akbari et al.° reported that preeclampsia is more likely in women, who have a vitamin D deficit of 20 ng/ml or less. At a threshold of 10.60 ng/ml, this connection may be 90% specific. This model has been used to assess the accuracy of the 25-OH vitamin D level in the diagnosis or prediction of preeclampsia. They also discovered two cutoffs of 10.60 and 20.05 ng/ml based on this. A cutoff of 9.79 has a specificity of up to 100%. This specificity was measured against the control groups and not against other illnesses and disorders. Furthermore, clinically, this 100% specificity will not occur as confidence intervals should be considered.

5. Conclusion

When preeclampsia pregnant women were compared with normotensive pregnant women, serum vitamin D values were considerably reduced. This research has suggested the potential for a link between maternal VDD and the prevalence of preeclampsia. Early identification of vitamin D insufficiency may help avoid preeclampsia and its consequences.

Consent statement

It was approved by the faculty Ethical Committee in 2017 and Al-Azhar International Medical Journal. It was accepted on 03/08/2022.

Conflict of interest

There are no conflicts of interest.

References


