

## RESEARCH

# Serum 25-hydroxyvitamin D levels throughout pregnancy: a longitudinal study in healthy and preeclamptic pregnant women

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## Abstract

**Background:** Worldwide there is a high prevalence of 25-hydroxyvitamin D (25OHD) deficiency and has been associated with adverse outcomes during pregnancy.

**Objective:** This is a nested, case-control study in a longitudinal cohort to compare the serum 25OHD levels and other biomarkers throughout pregnancy in a group of 20 preeclamptic women and 61 healthy pregnant women. An additional group of 29 healthy non-pregnant women were also studied during the two phases of the menstrual cycle.

**Results:** Mean 25OHD levels in non-pregnant women were 31.9 ng/mL and 34.9 ng/mL during follicular and luteal phase, respectively ( $P < 0.01$ ). Mean serum 25OHD levels in healthy pregnant women were 26.5, 30.1 and 31.9 ng/mL, at first, second and third trimester, respectively ( $P < 0.001$ ). The first trimester levels of 25OHD were lower than those of healthy non-pregnant women ( $P < 0.001$ ), showing a significant recovery at third trimester. In the group of healthy pregnant women, the 25OHD levels were 25.7 ng/mL and 27.2 ng/mL at 3 and 6 months postpartum, respectively; both values were lower than those observed in the non-pregnant women ( $P < 0.001$ ). In preeclamptic women, 25OHD serum levels were similar to those of healthy pregnant women; nevertheless, they remained almost unchanged throughout pregnancy.

**Conclusion:** There were no significant differences between healthy and preeclamptic pregnant women in terms of 25OHD levels throughout the pregnancy. Serum 25OHD levels in non-pregnant women were higher during luteal phase compared with follicular phase. The 25OHD levels of non-pregnant women tended to be higher than those of pregnant women.

### Key Words

- ▶ 25 hydroxyvitamin D
- ▶ pregnancy
- ▶ preeclampsia
- ▶ menstrual cycle

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## Introduction

There is a high prevalence of 25-hydroxyvitamin D (25OHD) deficiency during pregnancy (1). In the United States, a prevalence of 33% has been reported, while in

countries such as Turkey, the prevalence reaches 90% (1). 25OHD deficiency has been associated with adverse outcomes such as preeclampsia (2). This association is

supported by the fact that pregnant women with low serum 25OHD levels could have an increased activity of the renin–angiotensin–aldosterone system; an increase in pro-inflammatory cytokines and a decrease in the calcium absorption (3). Additionally, leptin has been shown to be a hormone that rises significantly during preeclampsia, and several authors in observational studies have described a correlation between elevated serum leptin levels and low serum 25OHD levels in the general population (4).

Moreover, Zabul *et al.* proposes that vitamin D3 might act as a competitive inhibitor of placental cytochrome P450<sub>scc</sub> preventing the production of lipid peroxides or excess of progesterone synthesis, both of which may contribute to the etiopathogenesis of preeclampsia (5).

The World Health Organization does not recommend the universal screening of serum 25OHD levels during pregnancy but in their vitamin D supplementation guidelines, they suggest that when the 25OHD levels are less than 20 ng/mL, supplementation should be provided (6).

The objective of this study is to compare the serum 25OHD levels in a group of healthy pregnant women during the first, second and third trimester of pregnancy, as well as 3 and 6 months postpartum, with a group of pregnant women with preeclampsia. In addition, serum 25OHD levels were determined in healthy non-pregnant women in the follicular and luteal phases of the menstrual cycle. Serum 25OHD levels were correlated with anthropometric, biochemical and hormonal parameters, such as leptin.

## Methods

### Patients and study design

This study was approved by the Ethics Committee of the School of Medicine of the Universidad Nacional de Colombia and was conducted according to the Helsinki Declaration. Patient's recruitment was performed by the Department of Obstetrics and Gynecology and Physiology of the School of Medicine of the Universidad Nacional de Colombia at the Hospital de Engativá in Bogotá Colombia, between January 2012 and December 2014. All patients in the study signed an informed consent.

In this nested case–control study in a longitudinal cohort of 450 pregnant women, 81 were included. In this cohort, pregnant women were recruited before the 14 weeks of gestation and the inclusion criteria were normal single pregnancy, BMI between 18 kg/m<sup>2</sup> and 30 kg/m<sup>2</sup>.

The exclusion criteria were the presence of any important medical comorbidity such as history of diabetes mellitus, gestational diabetes, past or present history of thyroid disease, vascular disease, chronic hypertension, renal disease, polycystic ovary syndrome, use of corticosteroids,  $\beta$ -blockers,  $\beta$ -agonists and other drugs that could affect metabolism.

During the pregnancy (since 14th week of gestational age), they were provided supplements as recommended by the Colombian Guidelines: carbonate calcium 600 mg 2 times per day, folic acid 1 mg per day and ferrous sulfate 300 mg per day (7).

Additionally, in the present study, the calcium supplementation provided to pregnant women was not adjusted to calcium status or nutrition and the calcium intake of the pregnant women was unknown.

Twenty pregnant women out of 450 developed preeclampsia (cases) and 61 healthy pregnant women were selected as controls.

The group of healthy pregnant women had no medical or obstetric complications during pregnancy or delivery and was followed during the three trimesters of pregnancy as well as 3 and 6 months postpartum.

Preeclampsia was defined by the criteria of the American College of Gynecology and Obstetrics, these criteria include features such as blood pressure  $\geq 140/90$  mmHg, examined twice with an interval of at least 4 h after 20 weeks of gestation (in a woman with previously normal blood pressure) and proteinuria  $>300$  mg by collection of 24-h urine (8). Severity features include thrombocytopenia, impaired liver function, progressive renal insufficiency, pulmonary edema, new-onset cerebral or visual disturbances (8). In this study, pregnant women with preeclampsia without severity features were studied.

Additionally, a group of 29 healthy non-pregnant women with regular menstrual cycles, without use of hormonal contraceptive methods, medications or supplements was recruited from the Universidad Nacional de Colombia. Serum 25OHD levels and other biochemical parameters were studied during the follicular (days 3–5) and luteal (days 20–22) phases of their menstrual cycle.

### Laboratory test

Blood samples were collected in 5.0 mL BD Vacutainer serum tubes from a forearm vein between 07:00 h and 08:00 h after an overnight fasting. The blood samples were centrifuged at 3500 g for 10 min at 4°C. Serum samples

were immediately transferred and stored in aliquots at  $-80^{\circ}\text{C}$  until biochemical and hormonal analysis were performed. The 25OHD-deficient pregnant women were not treated because the biochemical analyses were carried out long after the patients completed their pregnancy.

### Serum leptin levels measurement

Serum leptin levels were quantified using a commercially available Human ELISA Kit, according to the manufacturer's recommendations (Invitrogen, catalog number KAC2281). The intra-assay and inter-assay variation coefficients were  $<3.9$  and  $<5.3\%$ , respectively, and the sensitivity of the assay was less than  $<3.5$  pg/mL and the leptin ELISA kit shows no cross-reactivity with other cytokines tested. Also, serum leptin concentrations were analyzed in duplicate for each patient.

### Serum 25OHD levels measurement

Serum 25OHD levels were quantified by chemiluminescence immunoassay by commercially available kit, according to the manufacturer's protocols (LIAISON 25 OH Vitamin D TOTAL Assay REF 310600).

### Statistical analysis

Statistical analysis was performed using software Python with Scipy and Matplotlib libraries. The normality of the variables was defined by Kolmogorov–Smirnov test. Data with normal distribution were reported as mean  $\pm$  standard deviation (s.d.), while data with non-normal distribution were reported as median and interquartile range (IQR). Statistical differences between the anthropometric, hormonal and biochemical variables were established with non-parametric tests: Friedman test, Wilcoxon or Mann–Whitney.

We also studied the correlation between 25OHD and leptin levels. Univariate correlations were assessed in the groups by Spearman's partial correlation coefficient. Statistical values are presented as  $*P < 0.05$ ,  $**P < 0.01$  and  $***P < 0.001$ . Statistical significance was assumed with  $P < 0.05$ .

## Results

### Non-pregnant women

Twenty-nine non-pregnant women were characterized. Samples were obtained during the follicular and luteal

phases of the menstrual cycle and confirmed by measuring serum progesterone that was statistically different between the two phases (Table 1). Non-pregnant women had normal weight and normal blood pressure, and serum leptin levels were lower during the follicular phase of the menstrual cycle as shown in (Table 1).

The serum 25OHD levels changed during the menstrual cycle and were lower in the follicular phase compared with the luteal phase (31.9 ng/mL ( $\pm 6.0$ ) vs 34.9 ng/mL ( $\pm 8.6$ ),  $P < 0.01$ ) (Table 1).

### Healthy pregnant women

Sixty-one healthy pregnant women were characterized (Table 2). Mean serum 25OHD levels in healthy pregnant women were 26.5 ng/mL ( $\pm 5.27$ ), 30.1 ng/mL ( $\pm 8.1$ ) and 31.9 ng/mL ( $\pm 8.6$ ), at first, second and third trimester, respectively ( $P < 0.001$ ) (Table 2).

When serum 25OHD levels were compared between the first and second trimester of healthy pregnancy, a statistically significant increase was observed (26.5 ng/mL ( $\pm 5.3$ ) vs 30.1 ng/mL ( $\pm 8.1$ ),  $P < 0.001$ ), as well as a statistically significant increase was observed between the first and third trimester (26.5 ng/mL ( $\pm 5.3$ ) vs 31.9 ng/mL ( $\pm 8.6$ ),  $P < 0.001$ ) (Fig. 1 and Table 3).

Also, serum 25OHD levels were lower in the first trimester of the healthy pregnant women compared to the levels of non-pregnant women in both phases of menstrual cycle (Fig. 2 and Table 4).

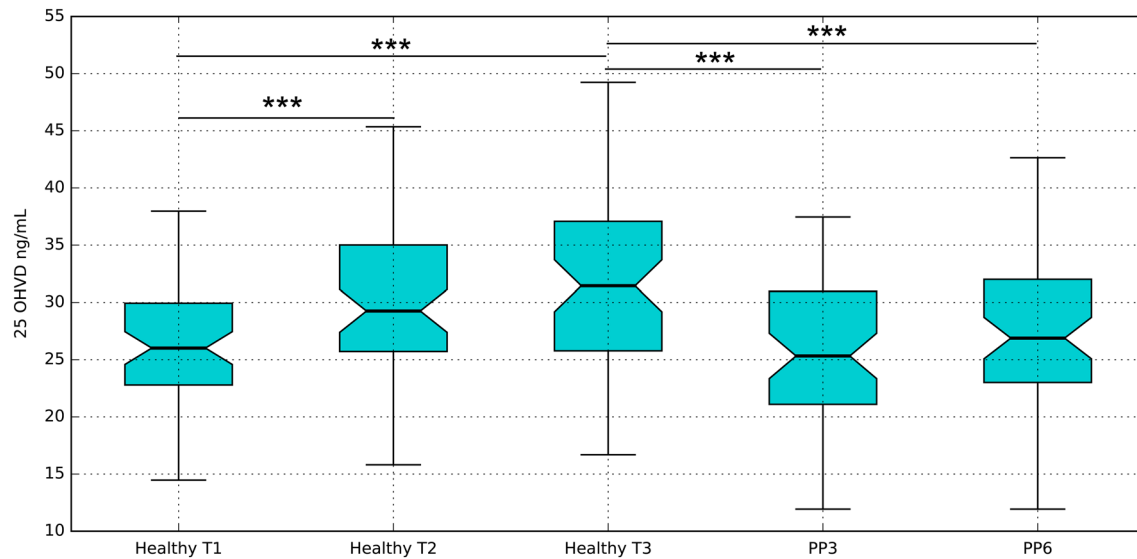
**Table 1** Non-pregnant women characteristics.

Non-pregnant women characteristics	Follicular phase (n=29)	Luteal phase (n=29)	P value
Age (years) median (IQR)	21 (18–22)	NA	NA
BMI (kg/m <sup>2</sup> ) mean $\pm$ s.d.	21.9 $\pm$ 2.0	NA	NA
SBP (mmHg) mean $\pm$ s.d.	108 $\pm$ 7	NA	NA
DBP (mmHg) mean $\pm$ s.d.	72 $\pm$ 7	NA	NA
Progesterone (ng/mL) median (IQR)	0.5 (0.3–0.7)	7.1 (4.7–11.1)	$<0.001$
Leptin (ng/mL) median (IQR)	15.7 (14.9–17.5)	22.3 (21.0–27.3)	$<0.001$
25OHD (ng/mL) mean $\pm$ s.d.	31.9 $\pm$ 6.0	34.9 $\pm$ 8.6	$<0.01$

The non-normal variables indicated as median (IQR), the variables with normal distribution are indicated as mean  $\pm$  s.d.

DBP, diastolic blood pressure; NA, non-applicable; SBP, systolic blood pressure.





**Figure 1**

Serum 25OHD levels of healthy pregnant women. T1, healthy first trimester; T2, healthy second trimester; T3, healthy third trimester. PP3, postpartum 3 months; PP6, postpartum 6 months. \**P* value <0.05, \*\**P* value <0.01, \*\*\**P* value <0.001.

of pregnancy, the frequency of 25OHD levels <30 ng/mL was 33/61, and for levels <20 ng/mL was 6/61. In the third trimester, the frequency of 25OHD levels <30 ng/mL was 26/61, and for levels <20 ng/mL was 5/61. At 3 months postpartum, the frequency of levels <30 ng/mL was 45/61 and for levels <20 ng/mL was 14/61.

Finally, at 6 months postpartum, the frequency of levels <30 ng/mL was 39/61 and for levels <20 ng/mL was 8/61.

## Discussion

The strengths of this study include the longitudinal design. Also, to the best of our knowledge, this is the first report of vitamin D status in Colombian pregnant women and in non-pregnant woman in both phases of menstrual cycle as controls. Additionally, Colombia is a tropical country without seasons due the geographical location (4° North and 74° West).

In this study, the serum 25OHD levels in non-pregnant women were lower in the follicular phase than in the luteal phase of menstrual cycle. Also, the 25OHD levels during the first trimester of healthy pregnancy were lower compared to non-pregnant women regardless of the menstrual cycle phase.

During the first trimester of healthy pregnant women, the 25OHD levels were lower compared to second and third trimester of pregnancy. And serum 25OHD levels did not change significantly during pregnancy complicated by

preeclampsia, and in general, there were no statistically significant differences between preeclamptic and healthy pregnant women.

Previous studies have indicated that vitamin D do not change during menstrual cycle phases (9, 10). However, in those studies, the sample size was small, Muse *et al.* study ( $n=6$ ) and Nielsen *et al.* study ( $n=8$ ) (9, 10). Other studies have described that there is a variation in calcium-related hormones, especially in 1,25(OH)<sub>2</sub>D during menstrual cycle, but these studies also have limitation in sample size (11, 12, 13, 14). Franasiaik *et al.* recently published a study that included 33 patients and reported that total and free 25OHD levels do not change throughout the follicular phase of the menstrual cycle, but 25OHD was not determined in the luteal phase (15). The authors textually conclude: 'Future areas of study should more fully explore the luteal phase changes to determine if alterations in vitamin D metabolism might become apparent' (15).

In addition, Refaat *et al.* showed a higher expression of vitamin D metabolic and signaling molecules such as vitamin D receptor (VDR), retinoid x receptor (RXR), calcium sensing receptor (CasR) in luteal phase than in the follicular phase of menstrual cycle in human fallopian tubal epithelial cells from non-pregnant women with normal serum 25OHD levels (>32 ng/mL) (16).

In the present study, a significant difference in serum 25OHD levels was found between both phases of the menstrual cycle. A physiological mechanism that could explain it, would be the influence of estrogen in follicular phase on renal 1 alpha hydroxylase, which would



**Table 3** Serum 25OHD levels of healthy pregnant women and postpartum.

Variable	Healthy pregnant women and postpartum (n=61)				*F	Post hoc analysis P value					
	T1	T2	T3	PP6		PP3	PP6	T1 vs T2	T2 vs T3	T3 vs T3	PP3 vs PP6
25OHD (ng/mL)	26.5 ± 5.3	30.1 ± 8.1	31.9 ± 8.6	25.7 ± 6.7	27.2 ± 6.8	<0.001	<0.001	0.105	<0.001	<0.001	0.173

The variables with normal distribution are indicated as mean ± s.d.  
 \*F, Friedman test; PP3, postpartum 3 months; PP6, postpartum 6 months; T1, healthy first trimester; T2, healthy second trimester; T3, healthy third trimester.

increase the conversion of 25OHD to 1,25(OH)<sub>2</sub>VD by the kidney (17).

Nonetheless, several authors have associated progesterone with inhibition of 24 hydroxylase, the function of this enzyme is to metabolize 25OHD and 1,25(OH)<sub>2</sub>D to inactive metabolites, moreover the excess in 1,25(OH)<sub>2</sub>D inhibits 1 alpha hydroxylase (18).

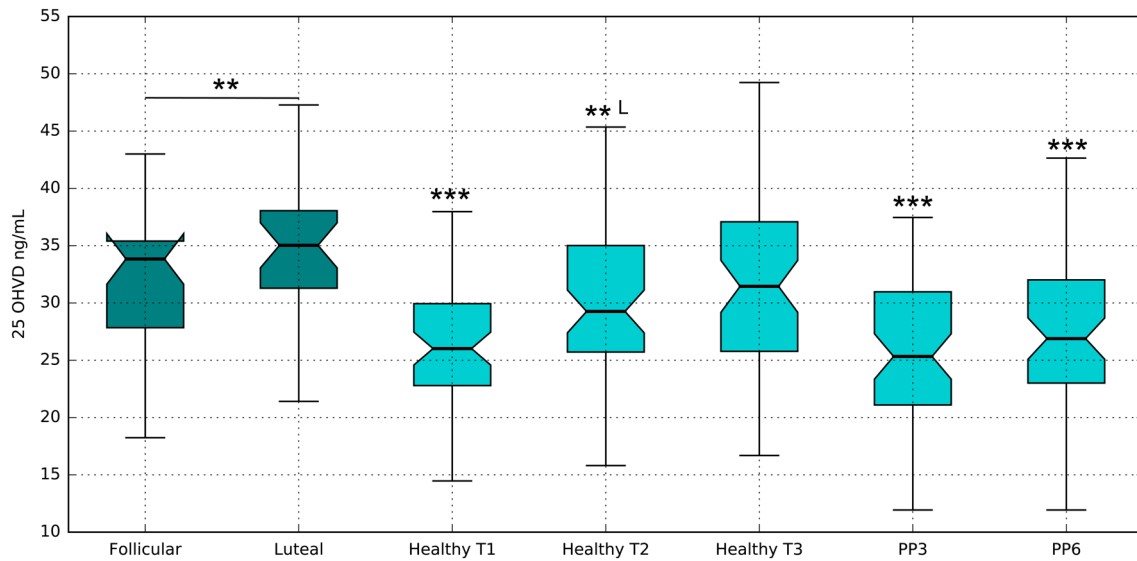
From this point of view, the circulating serum 25OHD levels would be decreased in the follicular phase because of the increase of their metabolism. However, further studies with a large sample size are needed due to the conflicting evidence around 25OHD during menstrual cycle.

The observation that 25OHD levels during the first trimester in healthy pregnant women are lower than those of non-pregnant women has previously been described (19, 20, 21, 22). Holmes *et al.* found that serum 25OHD levels were lower in pregnant women (n=98) compared to non-pregnant women (n=38) (P<0.0001) (18). Even, Khan *et al.* have found a higher prevalence of vitamin D deficiency in pregnant women (n=36) than in non-pregnant women (n=35) despite being matched by age and same area of residence (20). Also, Tamblyn *et al.* found lower levels of 25OHD in healthy pregnant women (n=25) than in non-pregnant women (n=9) (21). Moreover, Gellert *et al.* showed that 25OHD levels were lower in pregnant women in all trimesters of pregnancy (n=429) compared to non-pregnant women (n=429) and found an odds ratio (OR) of 13 to develop vitamin D deficiency in pregnant women compared to non-pregnant women (n=26) (22).

In all those studies, as well as in ours, the non-pregnant women and the pregnant ones were different. Therefore, the non-pregnant women could be in different nutritional and socio-economic conditions.

This is the first publication, to our knowledge, that includes non-pregnant women in both phases of the menstrual cycle. This study shows that serum 25OHD levels were lower in the first trimester of healthy pregnancy compared to non-pregnant women (regardless of the phase of menstrual cycle). A possible explanation would be the highest conversion rate of 25OHD to 1,25(OH)<sub>2</sub>VD as a consequence of an increased 1 alpha hydroxylase activity in the maternal kidney (23, 24). This increased activity of 1 alpha hydroxylase could be due the induction mediated by estrogen, prolactin and insulin growth factor 1 (25, 26, 27, 28).

On the other hand, the serum 25OHD levels were lower in the first trimester of healthy pregnancy compare to the two late trimester of pregnancy. This finding has been described not only in our study but also by



**Figure 2**

Serum 25OHD levels of healthy pregnant women compared to non-pregnant women. T1, healthy first trimester; T2, healthy second trimester; T3, healthy third trimester. PP3, postpartum 3 months; PP6, postpartum 6 months. \*\*L, the levels were statistically significant different only with the luteal phase. The other asterisks indicate statistically significant difference for the comparison with both phases of the menstrual cycle \*\*P value <0.01, \*\*\*P value <0.001.

other authors (29, 30, 31). While other studies have shown contrary results such as the study of Zhang *et al.* in which serum 25OHD levels were lower in the third trimester than in the first trimester (32).

The available evidence regarding maternal 25OHD concentration in pregnancy is conflicting, probably due ultraviolet light, lifestyle habits and genetic polymorphisms in the vitamin D receptor gene (*VDR*) and the vitamin D-binding protein gene (*DBP*) as other authors have discussed before (29).

Therefore, the significant increase in serum 25OHD levels throughout healthy pregnancy need to be explored in future studies. However, it would be interesting to consider the eventual effect of progesterone, a hormone that increases during pregnancy (33). Progesterone has an inhibitory role on 24-hydroxylase and could maintain circulating levels of 25OHD and 1,25(OH)<sub>2</sub>D for a longer time. Moreover, elevated serum levels of 1,25(OH)<sub>2</sub>VD inhibits the 1-alpha hydroxylase, which decrease the conversion of the substrate 25OHD to

1,25(OH)<sub>2</sub>D (17). Additional studies are required to corroborate the progesterone effects over the 25OHD levels during pregnancy.

With respect to preeclampsia, serum 25OHD levels did not change significantly throughout pregnancy and did not differ from those of healthy pregnant women.

Other authors reported low serum 25OHD levels in preeclamptic pregnant women (2, 34). Even, Wei *et al.* describe patients with low serum 25OHD levels during the second trimester of pregnancy present an OR of 3.24 (95% confidence interval CI, 1.37–7.69) to develop preeclampsia (35).

Also, Zhao *et al.*, in a large sample of pregnant women, found that low serum 25OHD levels during the second trimester of pregnancy have an OR 3.16 (95% CI, 1.77–5.65) to develop preeclampsia (36).

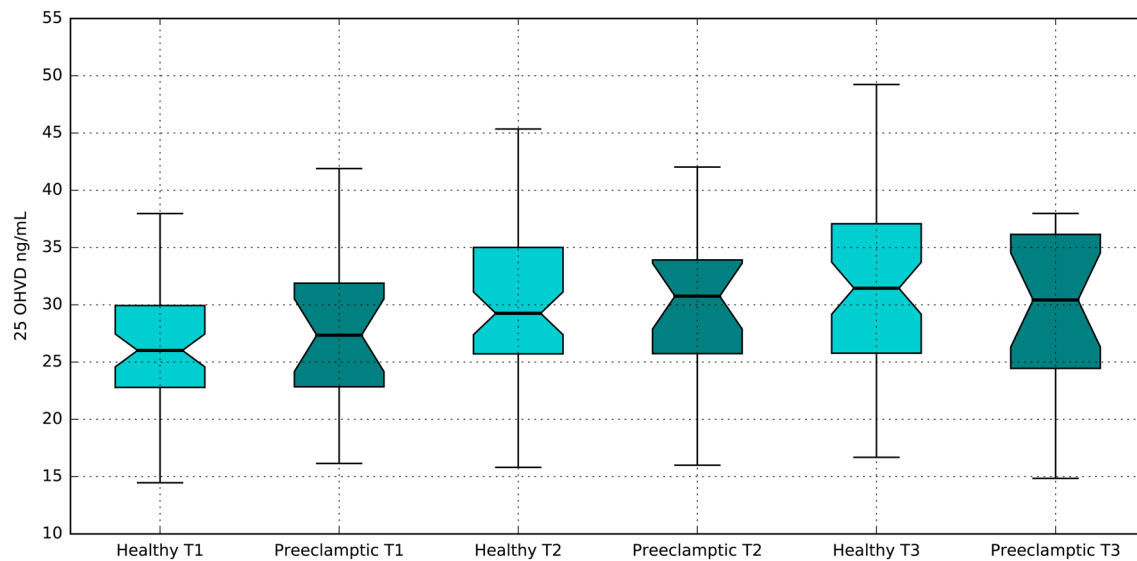
The contrasting results may be due to the fact that the patients included in the present study were only those with non-severe preeclampsia and the sample size may interfere with the results. However, Tamblyn *et al.*

**Table 4** Serum 25OHD levels in healthy pregnant women compared to non-pregnant women.

25OHD ng/mL non-pregnant women (n=29)	25OHD ng/mL healthy pregnant women and postpartum (n=61)				
	T1: 26.5±5.3	T2: 30.1±8.1	T3: 31.9±8.6	PP3: 25.7±6.7	PP6: 27.2±6.8
Follicular 31.9±6.0	P<0.001	P=0.12	P=0.79	P<0.001	P<0.001
Luteal 34.9±8.6	P<0.001	P<0.01	P=0.12	P<0.001	P<0.001

The variables with normal distribution are indicated as mean±s.d.

PP3, 3 months postpartum; PP6, 6 months postpartum; T1, healthy first trimester; T2, healthy second trimester; T3, healthy third trimester.

**Figure 3**

Serum 25OHD levels of health pregnant women compared to preeclamptic women. T, trimester. Comparisons among preeclamptic women: Friedman test=1, Analysis *post hoc* (preeclamptic T1 vs preeclamptic T2)  $P=0.47$ ; (preeclamptic T2 vs preeclamptic T3)  $P=1$ ; (preeclamptic T1 vs preeclamptic T3)  $P=0.71$ .

in a similar sample size also did not find a statistically significant difference in serum 25OHD levels between healthy and preeclamptic pregnant women (23).

Finally, the frequency in the first trimester of pregnancy of serum 25OHD levels lower than 30 ng/mL (Endocrine Society cutoff point for inadequate serum 25OHD levels) was 46/61. Additionally, the frequency of levels lower than 20 ng/mL (cut-off point IOM for inadequate serum 25OHD levels) was 6/61. These data are similar to those reported in Brazil by Figueiredo *et al.* who reported a prevalence of levels below 30 ng/mL around 70.4% and levels below 20 ng/mL of 16.1% (37).

In contrast to these results, other countries such as Turkey reported a prevalence of serum 25OHD levels below 20 ng/mL in about 90% (1).

The limitations of this study include a small sample size and lack of data about sun exposure and food intake. Those limitations may make results less generalizable to other population.

## Conclusion

Pregnant women are a population group with a higher risk of 25OHD insufficiency. There were no significant differences between healthy and preeclamptic women in terms of 25OHD levels throughout pregnancy. Serum 25OHD levels in non-pregnant women were higher during luteal phase compared with follicular phase. Regardless

of the phase of menstrual cycle, the 25OHD levels of non-pregnant women tended to be higher than those of pregnant women. The differences were found during the first and second trimester, while there were no differences during the third trimester.

Finally, no correlation between 25OHD levels and leptin levels was found.

### Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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