PHARMACIA

Pharmacia 72: 1–7
DOI 10.3897/pharmacia.72.e159409

Research Article

Association between maternal vitamin D, calcium, and inflammatory cytokines in Iraqi pregnant women with placental calcification

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Received 22 May 2025 ♦ **Accepted** 23 October 2025 ♦ **Published** 6 November 2025

Citation: Majeed Hashim Z, Kaisar Fakree N, Ali Abid Al-Juboori A, Karim MdF, Mohammed Radhi Z, Ali Khalaf Z, Al-Gburi H, Sadiq Amin S, Hassan Alkutbi S, Sadiq Nassir E, Ismail Ibrahim A, Ismail Ibrahim Z, Ayed Hassan E, Ferdoushi A (2025) Association between maternal vitamin D, calcium, and inflammatory cytokines in Iraqi pregnant women with placental calcification. Pharmacia 72: 1–7. https://doi.org/10.3897/pharmacia.72.e159409

Abstract

Placental calcification is an aging process that is increasingly linked to adverse pregnancy outcomes when it occurs prematurely. Vitamin D has immunomodulatory properties and may play a role in placental calcification. This study explored the association between maternal vitamin D, inflammatory cytokines, and placental calcification. A total of 46 pregnant women aged 16–40 years were included in this study. They were divided into four groups: Group 1 (control group; n = 10, without placental calcification), Group 2 (n = 12, Grade 1 placental calcification), Group 3 (n = 12, Grade 2 placental calcification), and Group 4 (n = 12, Grade 3 placental calcification). Serum vitamin D (25(OH)D) and cytokine (TNF-α, IL-2, IL-4, IL-10) levels were measured by ELISA, while serum calcium levels were measured by colorimetric analysis. Vitamin D levels declined gradually with increasing placental calcification ($p = 0.0146$ for G3 vs. control). Calcium levels did not differ significantly across groups ($p > 0.05$). The pro-inflammatory marker TNF-α was significantly increased in G3 compared to the control group ($p = 0.016$). Similarly, IL-2 levels increased, but the difference was not statistically significant. In contrast, anti-inflammatory cytokines IL-4 and IL-10 decreased, with IL-10 showing a significant positive correlation with vitamin D levels ($r = 0.8403$, $p = 0.0006$). These findings suggest that vitamin D deficiency may play a significant role in placental calcification, mediated by elevated pro-inflammatory cytokine TNF-α and reduced anti-inflammatory cytokine IL-10.

Keywords

anti-inflammatory cytokines, calcium, placental calcification, pro-inflammatory cytokines, vitamin D

Introduction

The placenta has a crucial role in fetal development. It acts as the primary interface between the maternal and fetal systems. The placenta not only ensures nutrient and oxygen exchange but also regulates immune tolerance during pregnancy (Gude et al. 2004; Al-Juboori et al. 2019). Dysfunction of the placenta is associated with adverse pregnancy outcomes, such as fetal growth restriction and preeclampsia (Ortega et al. 2022). Placental calcification is considered a physiological process of aging, but it may contribute to disease development when it occurs prematurely. Ischemic damage, oxidative stress, and inflammatory responses further compromise placental function (Ortega et al. 2024).

Placental calcification is classified as dystrophic or metastatic. Dystrophic calcification results from tissue damage and ischemia, with mineral deposits forming in necrotic areas (Rduch et al. 2022). Metastatic calcification occurs due to systemic mineral imbalances resulting from calcium and phosphate metabolism (Ortega et al. 2024). Calcification typically increases beyond 36 weeks of gestation. Premature onset of this calcification, called preterm placental calcification, is associated with pregnancy complications, including fetal growth restriction, preeclampsia, and preterm birth (Chen et al. 2011; Fox et al. 2019).

In addition to affecting calcium and bone metabolism, vitamin D has a role in both maternal and fetal health (Zhang et al. 2022). Its functions range from immunomodulatory effects to innate and adaptive immune responses (Ghaseminejad-Raeini et al. 2023). Vitamin D deficiency during pregnancy is an emerging global concern, with a prevalence rate ranging from 18% to 84%, depending on geographic location and lifestyle factors (Al Emadi and Hammoudeh 2013). Besides its role in fetal skeletal development, insufficient vitamin D levels are linked to various complications, including preeclampsia, gestational diabetes mellitus, preterm labor, and fetal respiratory infections (Giourga et al. 2023).

Vitamin D may also have a role in trophoblast invasion, angiogenesis, and vascular remodeling (Parenti et al. 2024). The trophoblast-localized enzyme 1-hydroxylase (*CYP27B1*) converts 25-hydroxyvitamin D [25(OH)D] to its active form, 1.25-dihydroxyvitamin D [$1.25(OH)_2D_3$], which has paracrine and autocrine effects on the placenta (Bikle et al. 2018). The vitamin D receptor (*VDR*) in placental tissue regulates inflammatory pathways and immune responses during pregnancy (Cao et al. 2021; Abdulrahman and Ali 2024). Low maternal $25(OH)D_3$ levels are reported to increase placental inflammation, alter immune tolerance, and elevate the risk of adverse pregnancy outcomes (Mansur et al. 2022).

Generally, vitamin D shifts the immune response from a pro-inflammatory Th1 to an anti-inflammatory Th2 profile (Aranow 2011). Calcitriol, the active form of vitamin D, inhibits the proliferation and differentiation of T helper (Th) cells and modulates their cytokine secretion profile. Pro-inflammatory and anti-inflammatory cytokines are regulated in opposite directions: interleukin-2 (IL-2), interferon-γ (INF-γ), tumor necrosis factor-alpha (TNF-α), and interleukin-9 (IL-9) are downregulated, whereas interleukin-4 (IL-4), interleukin-5 (IL-5), and interleukin-10 (IL-10) are upregulated (Roffe-Vazquez et al. 2019). Elevated levels of TNF-α and IL-2 have been linked to inflammation-induced placental dysfunction, whereas IL-4 and IL-10 play protective roles (Carpentier et al. 2011; Raghupathy 2013; Chatterjee et al. 2014). This indicates that maternal vitamin D levels may influence placental inflammation, thereby inducing calcification. The present study explores the relationship between maternal vitamin D status and inflammatory cytokine levels, particularly IL-2, TNF-α, IL-4, and IL-10, and placental calcification.

Materials and methods

Study population and design

The study enrolled 46 pregnant women aged 16–40 years (mean ± SE = 28.4 ± 6.3) who attended the Departments of Obstetrics and Gynecology and Radiodiagnosis at Al-Yarmouk Teaching Hospital, Baghdad, Iraq, between March and August 2024. Gestational age was confirmed using both the last menstrual period and ultrasound biometry.

Detailed obstetric and clinical histories were collected through structured interviews and medical record reviews. Information included parity (first, second, or third pregnancy), pre-pregnancy body mass index (BMI), blood pressure, and any previous pregnancy complications such as preeclampsia or preterm labor. All participants were nonsmokers and reported no use of alcohol or psychoactive substances. None had received vitamin D or calcium supplementation, corticosteroids, hormonal therapy, or any chronic medication within the past 3 months.

This recruitment approach was designed to minimize potential confounding factors such as medication use, metabolic or hypertensive disorders, and lifestyle variations that could influence serum vitamin D and cytokine levels.

Grouping of study participants

Based on ultrasound grading, the study participants were categorized into four groups: Group 1 (control; n = 10, pregnant women without placental calcification), Group 2 (n = 12, Grade 1 placental calcification), Group 3 (n = 12, Grade 2 placental calcification), and Group 4 (n = 12, Grade 3 placental calcification). According to ultrasound findings, Grade 1 (G1) showed random echogenic areas within the placenta; Grade 2 (G2) demonstrated echogenic densities along the basal plate with indentations in the chorionic plate; and Grade 3 (G3) represented the advanced stage, with a combination of echo-poor areas, irregular echogenic regions, and deep indentations in the chorionic plate (Grannum et al. 1979).

Inclusion and exclusion criteria

Inclusion criteria

Singleton pregnancies between 28 and 40 weeks of gestation, normal fetal anatomy confirmed by ultrasound, absence of maternal systemic disease, and willingness to provide written informed consent.

Exclusion criteria

Pregnant women with any maternal or fetal condition that could interfere with vitamin D metabolism, inflammatory status, or placental function were excluded. Specifically, women were excluded if they had a history of diabetes mellitus, chronic hypertension, thyroid, renal, hepatic, or autoimmune diseases, or any diagnosed metabolic or endocrine disorder.

Participants were also excluded if they had multiple gestations, fetal malformations, intrauterine growth restriction, or any obstetric complications identified by ultrasound.

Women who had taken vitamin D or calcium supplements, corticosteroids, hormonal therapy, or any medication known to affect calcium–vitamin D homeostasis within the preceding 3 months were not eligible.

In addition, smokers and those reporting alcohol or psychoactive substance use were excluded to avoid confounding lifestyle effects. Participants with incomplete clinical records or who declined written informed consent were also excluded.

Ethical approval

The study protocol was approved by the Ethical Committee of the College of Pharmacy, University of Baghdad, Iraq (RECOS2024109H). Before sample collection, written informed consent was obtained from all participants.

Blood sample collection and biochemical analysis

Five milliliters of fasting venous blood was collected from each participant into a Vacutainer tube. The collected blood samples were centrifuged at 3,000 rpm for 10 minutes. The serum was then separated and stored at –80 °C for further analysis.

Serum vitamin D [25(OH)D] levels were evaluated using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Calbiotech, Cat. No. 220B, China) following the manufacturer's protocol. Serum calcium levels were measured using the Arsenazo III colorimetric method following the manufacturer's instructions (Quimica Clinica Aplicada, Spain). Cytokine levels of TNF-α, IL-2, IL-4, and IL-10 were quantified using ELISA kits (Elabscience, Cat. No. E-EL-H0109, E-EL-H0099, E-EL-H0101, and E-EL-H0103, respectively, China).

Statistical analysis

SPSS version 24.0 (IBM Corp., USA), Student's t-test, and Pearson's correlation coefficient (r) were used for statistical analysis. The significance threshold for the Student's t-test was set at $p < 0.05$. All results were expressed as mean ± standard error (SE).

Abbreviations

TNF-α	Tumor necrosis factor-alpha
IL	Interleukin
ELISA	Enzyme-linked immunosorbent assay
VDR	Vitamin D receptor
CYP27B1	1α-hydroxylase enzyme
PCOS	Polycystic ovary syndrome
RAS	Renin-angiotensin system
SE	Standard error

Results

Serum vitamin D and calcium levels in placental calcification groups

Serum vitamin D levels [25(OH)D] decreased significantly across all placental calcification grades compared with the control group (Tables 1, 2). Pregnant women with Grade 3 placental calcification showed the lowest vitamin D levels (8.78 ± 0.40 ng/mL), which were significantly lower than those in the control group (12.01 ± 1.23 ng/mL, $p = 0.0146$). Vitamin D levels progressively declined from Grade 1 (10.10 ± 1.11 ng/mL) to Grade 2 (9.37 ± 0.65 ng/mL) and Grade 3 (8.78 ± 0.40 ng/mL); however, the differences between intermediate grades were not statistically significant. In contrast, there was no significant variation in calcium levels between the control and placental calcification groups (Tables 1, 2). No significant differences were found in Grade 1 (9.07 ± 0.09 mg/mL, $p = 0.154$), Grade 2 (9.10 ± 0.08 mg/mL, $p = 0.227$), or Grade 3 (9.11 ± 0.05 mg/mL, $p = 0.130$). These results suggest that the association between placental calcification and vitamin D deficiency is stronger than that with serum calcium levels.

Table 1. Serum levels of vitamin D, calcium, and cytokines in pregnant women with different grades of placental calcification.

Group	Grade (Samples)	Vitamin D (ng/ml)	Calcium (mg/dL)	TNF-α (pg/ml)	IL-2 (pg/ml)	IL-4 (pg/ml)	IL-10 (pg/ml)
Gr 1	Control (Grade 0, n = 10)	12.01 ± 1.23	9.25 ± 0.06	18.86 ±2.56	777.13 ± 120.70	743.33 ± 207.14	44.11 ± 9.00
Gr 3	Grade 1 (n = 12)	10.10 ± 1.11	9.07 ± 0.09	26.05 ±2.90	737.56 ±151.32	487.35 ± 169.44	31.96 ± 5.52
Gr 2	Grade 2 (n = 12)	9.37 ± 0.65	9.10 ± 0.08	25.86 ±5.14	1152.84 ± 262.15	468.71 ± 89.03	27.99 ± 8.09
Gr 4	Grade 3 (n = 12)	8.78 ± 0.40	9.11 ± 0.05	38.72 ±6.53	1275.26 ± 226.49	539.01 ± 186.34	29.30 ± 7.65

Table 2. *p*-values for grade comparisons.

Comparison	Vitamin D	Calcium	TNF-α	IL-2	IL-4	IL-10
Control vs. Grade 1	0.266	0.154	0.084	0.844	0.348	0.247
Control vs. Grade 2	0.062	0.227	0.266	0.237	0.210	0.197
Control vs. Grade 3	0.0146*	0.130	0.016*	0.082	0.471	0.221
Grade 1 vs. Grade 2	0.577	0.799	0.975	0.183	0.917	0.688
Grade 1 vs. Grade 3	0.275	0.706	0.089	0.061	0.844	0.780
Grade 2 vs. Grade 3	0.450	0.937	0.136	0.727	0.736	0.907

* All values represent mean ± SE. Significant difference when $p < 0.05$.

Pro-inflammatory cytokine levels (TNF-α and IL-2) in placental calcification groups

Tumor necrosis factor-alpha (TNF-α) was significantly increased in the placental calcification groups (Tables 1, 2). Pregnant women with Grade 3 placental calcification showed significantly higher TNF-α levels (38.72 ± 6.53 ng/mL) compared with the control group (18.86 ± 2.56 ng/mL, $p = 0.016$). However, Grade 1 (26.05 ± 2.90 ng/mL, $p = 0.084$) and Grade 2 (25.86 ± 5.14 ng/mL, $p = 0.266$) showed no statistically significant differences compared with the control group. Interleukin-2 (IL-2) levels also increased in the placental calcification groups, but the difference was not statistically significant (Tables 1, 2). IL-2 levels were highest in Grade 3 (1275.26 ± 226.49 ng/mL) compared with the control group (777.13 ± 120.70 ng/mL, $p = 0.082$). There were no significant differences between Grade 1 (737.56 ± 151.32 ng/mL) and Grade 2 (1152.84 ± 262.15 ng/mL, $p > 0.05$).

Anti-inflammatory cytokine levels (IL-4 and IL-10) in placental calcification groups

In the placental calcification groups, there was a declining trend in the anti-inflammatory cytokines IL-4 and IL-10, consistent with the decrease in vitamin D levels (Tables 1, 2). IL-4 levels in the control group, Grade 1, Grade 2, and Grade 3 were 743.33 ± 207.14 ng/mL, 487.35 ± 169.44 ng/mL ($p = 0.348$), 468.71 ± 89.03 ng/mL ($p = 0.210$), and 539.01 ± 186.34 ng/mL ($p = 0.471$), respectively. Although IL-4 levels in Grades 1, 2, and 3 were lower than those in the control group, the differences were not statistically significant.

Similarly, IL-10 levels decreased progressively compared with the control group. IL-10 levels in the control group, Grade 1, Grade 2, and Grade 3 were 44.11 ± 9.00 ng/mL, 31.96 ± 5.52 ng/mL ($p = 0.247$), 27.99 ± 8.09 ng/mL ($p = 0.197$), and 29.31 ± 7.65 ng/mL ($p = 0.221$), respectively (Tables 1, 2). These results suggest a potential association between vitamin D deficiency and reduced anti-inflammatory cytokine production in placental calcification.

To provide a more precise comparison of key variables across the study groups, Fig. 1 summarizes the mean serum levels of vitamin D, TNF-α, and IL-10 for all grades of placental calcification. As shown in the figure, vitamin D and IL-10 levels progressively declined with increasing calcification grade. In contrast, TNF-α levels rose correspondingly, particularly in Grade 3, supporting the inflammatory trend observed in the quantitative analysis.

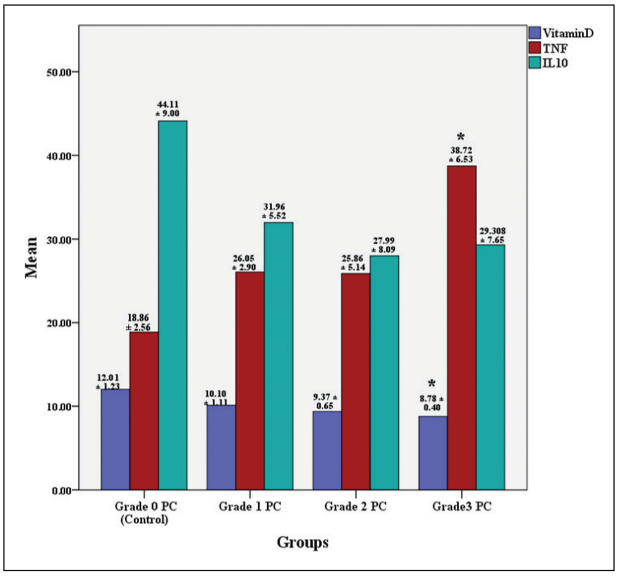

Figure 1. Comparative mean levels (± SE) of serum vitamin D, TNF-α, and IL-10 across placental calcification grades (control, Grade 1, Grade 2, and Grade 3). Asterisks indicate statistically significant differences versus the control group ($p < 0.05$).

Correlation between vitamin D and inflammatory cytokines

From the correlational analysis across different grades of placental calcification, as presented in Table 3, TNF-α showed a strong and significant negative correlation with vitamin D in Grade 1 (r = –0.80496, $p = 0.0016$), Grade 2 (r = –0.63097, $p = 0.0278$), and Grade 3 (r = –0.63793, $p = 0.0256$). These results indicate that TNF-α levels increase as vitamin D levels decrease, suggesting a possible role for inflammation in placental calcification. Interestingly, the control group (r = –0.33936, $p = 0.2529$) did not show a significant correlation, indicating lower inflammation in healthy pregnancies. In addition, IL-2 also demonstrated a negative correlation with vitamin D across all groups, though the association was not statistically significant ($p > 0.05$). This implies that while IL-2 levels may increase with placental calcification, their relationship with vitamin D is weaker than that of TNF-α. IL-4 showed a weak positive correlation with vitamin D ($p > 0.05$) across all groups, suggesting a limited vitamin D influence on IL-4. However, IL-10 demonstrated a strong and significant positive correlation with vitamin D in Grade 1 (r = 0.5838, $p = 0.0462$), Grade 2 (r = 0.8403, $p = 0.0006$), and Grade 3 (r = 0.6741, $p = 0.0162$), suggesting that higher vitamin D levels may regulate IL-10 expression

Table 3. Correlation between vitamin D levels and inflammatory cytokines.

Comparison	Vitamin D	Calcium	TNF-α	IL-2	IL-4	IL-10
Control vs. Grade 1	0.266	0.154	0.084	0.844	0.348	0.247
Control vs. Grade 2	0.062	0.227	0.266	0.237	0.210	0.197
Control vs. Grade 3	0.0146*	0.130	0.016*	0.082	0.471	0.221
Grade 1 vs. Grade 2	0.577	0.799	0.975	0.183	0.917	0.688
Grade 1 vs. Grade 3	0.275	0.706	0.089	0.061	0.844	0.780
Grade 2 vs. Grade 3	0.450	0.937	0.136	0.727	0.736	0.907

* Significant at $p < 0.05$; **highly significant at $p < 0.01$.

and counteract inflammation, thereby preserving placental function. There was no significant correlation in the control group (r = 0.4806, p = 0.1597), suggesting that IL-10 is not a major regulator in normal pregnancies.

Discussion

The novelty of this study lies in its insights into the association among maternal vitamin D levels, inflammatory cytokines, and placental calcification. This study found that vitamin D deficiency is significantly associated with increased placental calcification, especially in advanced cases. This is further supported by increased pro-inflammatory cytokine levels, notably TNF-α, and decreased anti-inflammatory cytokine levels, particularly IL-10, suggesting an inflammatory mechanism underlying placental calcification.

The results of this study are consistent with previous research demonstrating that vitamin D plays a crucial role in inflammation and immune regulation (Cyprian et al. 2019; Fakree et al. 2022; Zhang et al. 2022; Mhaibes and Abdul-Wahab 2023). Lower vitamin D levels were associated with greater calcification severity (p = 0.0146 for G3 vs. control), suggesting that vitamin D deficiency may promote placental dysfunction. The role of vitamin D status in placental inflammatory processes is further supported by the level of TNF-α, which is significantly negatively correlated with vitamin D (r = –0.804, p = 0.0016 for G1; r = –0.637, p = 0.0256 for G3). TNF-α is involved in various pathological processes, including trophoblast apoptosis, vascular dysfunction, and impaired nutrient exchange, contributing to placental calcification (Howell and Powell 2017; Mohammed et al. 2022; Mahde and Kathem 2023).

Interestingly, calcium levels did not differ significantly across groups (p > 0.05), suggesting a closer link between placental calcification and vitamin D status than between placental calcification and systemic calcium levels. This finding further indicates that placental calcification extends beyond simple calcium deposition and is a complex process regulated by multiple inflammatory pathways (Ortega et al. 2024).

While TNF-α levels increased, anti-inflammatory cytokines IL-4 and IL-10 showed a decreasing trend. IL-10 significantly correlated with vitamin D levels (r = 0.8403, p = 0.0006 for G2). IL-10 is essential for immune tolerance and suppresses excessive inflammation in the placenta (Cheng and Sharma 2015; Salman et al. 2022). These findings suggest a shift toward a pro-inflammatory state, further supporting vitamin D's role in controlling placental immunity.

Several studies demonstrate that IL-2 is upregulated in polycystic ovary syndrome (PCOS), which is also linked to vitamin D deficiency (Taher and Al-Juboori 2012; Mohan et al. 2023). In this study, IL-2 levels showed an increasing trend in the calcification groups, though the differences were not statistically significant. This indicates that IL-2 may play a secondary role in placental inflammation, whereas TNF-α might be the primary driver. The statistical insignificance of IL-2 changes could be due to the small sample size and individual variability.

The findings of this study have clinical importance. Given the strong correlation between vitamin D deficiency and inflammatory markers in placental calcification, vitamin D levels may serve as an additional biomarker for identifying high-risk pregnancies. Moreover, these findings suggest that vitamin D supplementation could be a potential therapeutic strategy to mitigate placental inflammation. It may also serve as a preventive mechanism against early placental calcification in populations at risk for vitamin D deficiency.

However, the findings of this study lack generalizability for healthcare policymaking because the sample size is relatively small (n = 46). Although a strong correlation among vitamin D deficiency, inflammation, and placental calcification was observed, establishing a causal relationship remains challenging. To explore the molecular mechanisms underlying placental calcification across different trimesters of pregnancy, it is necessary to develop in vitro models of placental calcification and compare them with in vivo calcified placental tissues using advanced omics technologies (Gude et al. 2004; Al-Juboori et al. 2019). Future longitudinal and interventional studies are warranted to determine whether vitamin D supplementation directly decreases placental calcification and improves pregnancy outcomes.

Conclusion

In conclusion, vitamin D deficiency and inflammatory cytokine imbalance are associated with placental calcification in Iraqi pregnant women. Decreased vitamin D levels, elevated TNF-α, and reduced IL-10 have been found in patients with advanced calcification. Vitamin D deficiency may play a vital role in the development of an inflammatory environment, leading to placental dysfunction. Interestingly, there were no significant differences in calcium levels, suggesting that systemic mineral levels may not be critical. It is thus plausible that placental calcification is associated with inflammatory and immunological consequences. The TNF-α level, a crucial cytokine in implantation and placental development, was elevated in Iraqi women with placental calcification, suggesting an inverse relationship. This unexpected TNF-α result may be attributed to the small sample size. Although this study involved a limited number of participants, its findings are essential for screening and correcting vitamin D deficiency during pregnancy as part of routine antenatal care. The results highlight that vitamin D supplementation could serve as a preventive strategy against early placental calcification. In the future, intervention studies are warranted to validate these findings and identify a healthcare strategy that integrates nutritional, immunological, and clinical approaches to improve placental function and pregnancy outcomes.

Acknowledgements

The authors sincerely thank the Department of Obstetrics and Gynecology and Radiodiagnosis at Al-Yarmouk Teaching Hospital, Baghdad, Iraq, for supporting participant recruitment and ultrasound assessments. The authors also acknowledge the College of Pharmacy, University of Baghdad, Iraq, for providing ethical approval and facilitating laboratory analyses. Special thanks go to the medical and technical staff who assisted in sample collection and biochemical analyses. Lastly, the authors thank the study participants for their cooperation and willingness to contribute to this research.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statements

The authors declared that no clinical trials were used in the present study.

The authors declared that experiments on humans or human tissues were performed for the present study.

The present research was approved by the Ethical Panel of the College of Pharmacy, University of Baghdad, Iraq (Approval No. RECOS2024109H). All procedures were conducted in accordance with the Declaration of Helsinki and the Guidelines for the Performance, Writing, Reviewing, and Publication of Scientific Research in Medical Journals. Informed consent was obtained from each participant before enrollment, and their privacy rights were strictly protected.

The authors declared that no experiments on animals were performed for the present study.

The authors declared that no commercially available immortalised human and animal cell lines were used in the present study.

Use of AI

No use of AI was reported.

Funding

This research received no specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Author contributions

Zainab M. Hashim contributed to funding acquisition, resources, methodology, conceptualization, and writing of the original draft. Najwan Kaisar Fakree participated in funding acquisition, resources, formal analysis, methodology, conceptualization, and writing of the original draft. Aminah Ali Abid Al-Juboori contributed to funding acquisition, statistical analysis, validation, methodology, conceptualization, and writing (review and editing), and served as the corresponding author. Md. Fazlul Karim provided supervision, project administration, validation, visualization, and writing (review and editing). Zahraa Mohammed Radhi assisted in data curation, investigation, and manuscript editing. Zeena Ali Khalaf contributed to sample collection, data curation, and laboratory analysis. Hasanain Al-Gburi participated in data interpretation and manuscript preparation. Seenaa S. Amin contributed to investigation, data management, and visualization. Suhair Hassan Ali assisted in methodology development, sample processing, and literature review. Eman Sadiq Nassir contributed to data validation, formal analysis, and figure preparation. Aseel I. Ibrahim participated in data collection, investigation, and manuscript editing. Zainab Ismail Ibrahim contributed to patient recruitment, data entry, and verification. Eman A. Hassan assisted in literature review, visualization, and manuscript editing. Aysha Ferdoushi provided overall supervision, project administration, validation, visualization, and writing (review and editing), and served as the co-corresponding author. All authors have read and approved the final version of the manuscript.

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Data availability

All of the data that support the findings of this study are available in the main text.

References

Abdulrahman NM, Ali SH (2024) Correlation of osteocalcin with vitamin D level in postmenopausal women concerning CYP24A1 and VDR gene polymorphisms. Al-Kindy Coll. Med. J. 20: 135–144. https://doi.org/10.47723/sdfrzk14

AlEmadi S, Hammoudeh M (2013) Vitamin D study in pregnant women and their babies. Qatar Med. J. 1: 32–37. https://doi.org/10.5339/qmj.2013.7

Al-Juboori AAA, Ghosh A, Bin Jamaluddin MF, Kumar M, Sahoo SS, Syed SM, Nahar P, Tanwar PS (2019) Proteomic analysis of stromal and epithelial cell communications in human endometrial cancer using a unique 3D co-culture model. Proteomics. 19: 21–22. https://doi.org/10.1002/pmic.201800448

Aranow C (2011) Vitamin D and the immune system. J Investig Med. 59(6): 881–886. https://doi.org/10.2310/jim.0b013e31821b8755

Bikle DD, Patzek S, Wang YDD (2018) Physiologic and pathophysiologic roles of extra renal CYP27b1: Case report and review. Bone Reports. 8: 255–267. https://doi.org/10.1016/j.bonr.2018.02.004

Cao Y, Jia X, Huang Y, Wang J, Lu C, Yuan X, Xu J, Zhu HY (2021) Vitamin D stimulates miR-26b-5p to inhibit placental COX-2 expression in preeclampsia. Sci. Rep. 11: 1–11. https://doi.org/10.1038/s41598-021-90605-9

Carpentier PA, Dingman AL, Palmer TD (2011) Placental TNF-α signaling in illness-induced complications of pregnancy. Am. J. Pathol. 178(6): 2802–2810. https://doi.org/10.1016/j.ajpath.2011.02.042

Chatterjee P, Chiasson VL, Bounds KR, Mitchell BM (2014) Regulation of the anti-inflammatory cytokines interleukin-4 and interleukin-10 during pregnancy. Front. Immunol. 5:253. https://doi.org/10.3389/fimmu.2014.00253

Chen KH, Chen LR, Lee YH (2011) Exploring the relationship between preterm placental calcification and adverse maternal and fetal outcome. Ultrasound Obstet. Gynecol. 37(3): 328–334. https://doi.org/10.1002/uog.7733

Cheng SB, Sharma S (2015) Interleukin-10: A pleiotropic regulator in pregnancy. Am J Reprod Immunol. 73(6): 487–500. https://doi.org/10.1111/aji.12329.

Cyprian F, Lefkou E, Varoudi K, Girardi G (2019) Immunomodulatory effects of vitamin D in pregnancy and beyond. Front. Immunol. 10: 2739. https://doi.org/10.3389/fimmu.2019.02739

Fakree NK, Hashim ZM, Muhsen AAS, Hashim NM. (2022) Some pro and anti-inflammatory cytokines in children with tonsillitis and their correlations with vitamin D deficiency. Iraqi J. Pharm. Sci. 31(1): 194–201. https://doi.org/10.31351/vol31iss1pp194-201

Fox R, Kitt J, Leeson P, Aye CYL, Lewandowski AJ (2019) Preeclampsia: Risk factors, diagnosis, management, and the cardiovascular impact on the offspring. J. Clin. Med. 8(10): 1652. https://doi.org/10.3390/jcm8101625

Ghaseminejad-Raeini A, Ghaderi A, Sharafin A, Nematollahi-Sani B, Moossavi M, Derakhshani A, Sarab GA (2023) Immunomodulatory actions of vitamin D in various immune-related disorders: a comprehensive review. Front. Immunol. 14: 950465. https://doi.org/10.3389/fimmu.2023.950465

Giourga C, Papadopoulou SK, Voulgaridou G, Karastogiannidou C, Giaginis C, Pritsa (2023) Vitamin D deficiency as a risk factor of preeclampsia during pregnancy. Disease 11 (4): 158. https://doi.org/10.3390/diseases11040158

Grannum PA, Berkowitz RL, Hobbins JC (1979) The ultrasonic changes in the maturing placenta and their relation to fetal pulmonic maturity. Am. J. Obstet. Gynecol. 133(8): 915–922. https://doi.org/10.1016/0002-9378(79)90312-0

Gude NM, Roberts CT, Kalionis B, King RG (2004) Growth and function of the normal human placenta. Thromb. Res. 114(5): 397–407. https://doi.org/10.1016/j.thromres.2004.06.038

Howell, KR, Powell TL (2017) Effects of maternal obesity on placental function and fetal development. Reproduction 153: 97–108. https://doi.org/10.1530/REP-16-0495

Mahde S, Kathem SH (2023) Anti-inflammatory effect of L-carvone on lipopolysaccharide-induced acute lung injury. Iraqi J. Pharm. Sci. 32(1): 125–132. https://doi.org/10.31351/vol32iss1pp125-132

Mansur JL, Oliveri B, Giacoia E, Fusaro D, Costanzo PR (2022) Vitamin D: before, during and after pregnancy: effect on neonates and children. Nutrients 14(9): 1900. https://doi.org/10.3390/nu14091900

Mhaibes AM, Abdul-Wahab FK (2023) Possible effects of vitamin D3 and levofloxacin on selected hematology parameter of rats. Iraqi J. Pharm. Sci. 32: 74–84. https://doi.org/10.31351/vol32issSuppl.pp74-84

Mohammed SI, Zalzala MH, Gorial FI (2022) The effect of TNF-alpha gene polymorphisms at -376 G/A, -806 C/T, and -1031 T/C on the likelihood of becoming a non-responder to etanercept in a sample of Iraqi Rheumatoid Arthritis Patients. Iraqi J. Pharm. Sci. 31(2): 113–128. https://doi.org/10.31351/vol31iss2pp113-128

Mohan A, Haider R, Fakhor H, Hina F, Kumar V, Jawed A, Majumder K, Ayaz A, Lal PM, Tejwaney U, Ram N, Kazeem S (2023) Vitamin D and polycystic ovary syndrome (PCOS): a review. Annals of Medicine and Surgery 85(7): 3506–3511. https://doi.org/10.1016/j.tjog.2022.06.010

Ortega MA, Fraile-Martínez O, García-Montero C, Sáez MA, Álvarez-Mon MÁ, Torres-Carranza D, Álvarez-Mon M, Buján J, García-Honduvilla N, Bravo C, Guijarro LG, De León-Luis JA (2022) The pivotal role of the placenta in normal and pathological pregnancies: a focus on preeclampsia, fetal growth restriction, and maternal chronic venous disease. Cells 11(3): 568. https://doi.org/10.3390/cells11030568

Ortega MA, Pekarek T, De Leon-oliva D, Boaru DL, Fraile-martinez O, Garcia-Montero C, Bujan J, Pekarek L, Barrena-bl S, Gragera R, Rodriguez-Benitez P, Hernández-Fernández M, Lopez-Gonzalez L, Díaz-Pedrero R, Asúnsolo A, Álvarez-Mon M, García- Honduvilla N, Saez MA, De León-Luis JA, Bravo C (2024) Placental tissue calcification and its molecular pathways in female patients with late-onset preeclampsia. Biomolecules 14(10):1237. https://doi.org/10.3390/biom14101237

Parenti M, Melough MM, Lapehn S, MacDonald J, Bammler T, Firsick EJ, Choi HY, Derefinko KJ, Enquobahrie DA, Carroll KN, LeWinn KZ, Bush NR, Zhao Q, Sathyanarayana S, Paquette AG (2024) Associations between prenatal vitamin D and placental gene expression. J Nutr. 154(12): 3603–3614. https://doi.org/10.1101/2024.05.10.593571

Raghupathy R (2013) Cytokines as key players in the pathophysiology of preeclampsia. Med. Princ. Pract. 22: 8–19. https://doi.org/10.1159/000354200

Rduch T, Tsolaki E, El Baz Y, Leschka S, Born D, Kinkel J, Anthis AHC, Fischer T, Jochum W, Hornung R, Gogos A, Herrmann IK (2022) The role of inorganics in preeclampsia assessed by multiscale multimodal characterization of placentae. Front. Med. 9: 857529. https://doi.org/10.3389/fmed.2022.857529

Roffe-Vazquez DN, Huerta-Delgado AS, Castillo EC, Villarreal-Calderón JR, Gonzalez-Gil AM, Enriquez C, Garcia-Rivas G, Elizondo-Montemayor L (2019) Correlation of vitamin D with inflammatory cytokines, atherosclerotic parameters, and lifestyle factors in the setting of heart failure: A 12-month follow-up study. Int. J. Mol. Sci. 20(22): 5811. https://doi.org/10.3390/ijms20225811

Salman OD, Merdaw MAZ, Almaliky AA (2022) A novel single nucleotide polymorphism of interleukin-10 gene is linked to type 2 diabetes mellitus in iraqi patients with toxoplasmosis (Conference paper). Iraqi J. Pharm. Sci. 31: 1–8. https://doi.org/10.31351/vol31issSuppl.pp1-8

Taher MA, Al-Juboori AA (2012) Levels of some pro-inflammatory cytokines in pregnant women with polycystic ovary syndrome. Al Mustansiriyah J. Pharm. Sci.11: 150–162. https://doi.org/10.32947/ajps.v11i1.249

Zhang H, Wang S, Tuo L, Zhai Q, Cui J, Chen D, Xu D (2022) Relationship between maternal vitamin D levels and adverse outcomes. Nutrients 14(20): 4230. https://doi.org/10.3390/nu14204230