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RESEARCH ARTICLE

Wojczakowski et al: Vitamin D and calcium in PE and PIH

Vitamin D and calcium status in preeclampsia and pregnancy-induced hypertension

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ABSTRACT

Hypertensive disorders of pregnancy are major causes of maternal and perinatal morbidity and mortality, and nutritional factors such as vitamin D and calcium have been proposed as modifiable risks; therefore, we investigated the association between maternal vitamin D and calcium status and pregnancy-induced hypertension (PIH) and pre-eclampsia (PE) and explored the relation with supplementation. In this observational cross-sectional study, 84 third-trimester women were enrolled from two hospitals in Lublin, Poland (41 PIH/PE, 43 controls). Serum total and ionised calcium, 25-hydroxyvitamin D [25(OH)D], and 1,25-dihydroxyvitamin D_3 were measured using standardised immunoassays, and group differences, correlations, and multivariable logistic regression were applied with adjustment for body mass index (BMI), maternal age, gestational age, calcium fractions, and gestational diabetes. PIH/PE cases had lower 25(OH)D than controls (27.8 vs 35.7 ng/mL; $p = 0.012$) and higher BMI (33.0 vs 27.5 kg/m²; $p < 0.001$), while total and ionised calcium and 1,25-dihydroxyvitamin D_3 were similar (all $p \geq 0.40$); supplement use was more frequent among controls (84% vs 73%). In adjusted models, higher BMI increased the odds of PIH/PE (OR 1.19 per kg/m²) and higher 25(OH)D was protective (OR 0.92 per ng/mL); discrimination was fair (AUC 0.78). These findings support an association between vitamin D insufficiency and obesity with hypertensive pregnancy disorders and suggest preserved calcium homeostasis, but given the cross-sectional design, third-trimester sampling, small sample size, and non-standardised supplementation, causal inference and preventive recommendations cannot be made; larger prospective studies beginning in early pregnancy are warranted to test whether optimising vitamin D and calcium can reduce hypertensive complications.

Keywords: Vitamin D deficiency, calcium deficiency, hypertensive pregnancy.

INTRODUCTION

Clinical descriptions of eclampsia can be traced back over 2,400 years, whereas pre-eclampsia — formerly referred to as "toxicaemia of pregnancy" — was recognised as a distinct clinical syndrome only in the 19th century [1]. Although pre-eclampsia has been described for centuries, its pathophysiology is still not fully understood, which limits effective treatment options [1].

Pregnancy-induced hypertension (PIH) occurs in 3.6% to 9.1% of pregnancies, while pre-eclampsia affects 1.4% to 4.0% of pregnant women. Together, they contribute to significant maternal and perinatal morbidity and mortality, with pre-eclampsia accounting for over 70,000 maternal deaths and 500,000 foetal deaths annually worldwide [2]. Though less common, eclampsia is responsible for approximately 4.2% of maternal deaths and up to 30% of neonatal deaths [3].

Pre-eclampsia is associated with health problems for offspring [4], like a 1.3-fold increased risk of stillbirth and a 2-fold increased risk of neonatal death [5]. It is also a major cause of foetal growth restriction (FGR). Pre-eclampsia leads to babies being small for their gestational age in approximately 20% of full-term births and 60% of premature births [6]. It is an important cause of prematurity. A recent large multicenter study found that the proportion of premature babies among women with pre-eclampsia is 36% [7] compared to about 10% in the general population [8]. Kajantie et al. showed an almost 2-fold increase in the risk of stroke in people whose mothers suffered from pre-eclampsia [9].

Apart from the mortality itself, the long-term consequences for the mother should also be mentioned. A meta-analysis published in 2007 that included around 200,000 cases of pre-eclampsia revealed relative risks of 3.7, 2.16, and 1.81 respectively for hypertension, ischaemic heart disease, and stroke, after a mean of 10–15 years of follow-up [10]. Women who had pregnancies with early-onset pre-eclampsia also had an increased risk of cardiovascular diseases (CVD) risk factors, including elevated fasting glucose, insulin, triglycerides, and total cholesterol, compared with women who experienced late-onset pre-eclampsia or pregnancy-induced hypertension [11].

Women with a history of pre-eclampsia are at greater risk of developing diabetes, even if they have not developed gestational diabetes [12]. They are also prone to developing chronic kidney disease and hypertensive kidney disease. They are five times as likely to develop end-stage renal disease as women without pre-eclampsia [13]. There is also an increased risk of developing neurological diseases, such as a 3-

fold higher likelihood of vascular dementia and a potentially increased chance of developing disorders of perception, memory, and motor function [14].

Nutritional factors such as calcium and vitamin D status have emerged as potentially modifiable risk factors. Up to 60% of women have insufficient blood levels of vitamin D (below 30 ng/ml) and calcium (below 9 mg/dl) [15]. Multiple randomised controlled trials and meta-analyses have shown that vitamin D supplementation during pregnancy reduces the risk of pre-eclampsia by around 40–60 % [16-19]. Similarly, calcium supplementation (≥ 1 g/day) cuts pre-eclampsia risk by about 50 %, with both low- and high-dose regimens being effective, particularly among women with low baseline calcium intake [20-22]. The fundamental problem, despite the extensive literature, remains the assessment of vitamin D concentrations that would reduce the risk of developing pre-eclampsia PE [23].

MATERIALS AND METHODS

The present study is an observational study primarily aimed at determining the association between serum levels of vitamin D and calcium and the development of pregnancy-induced hypertension and pre-eclampsia in pregnant women. A secondary objective was to assess the effect of vitamin D and calcium supplementation on reducing the risk of these conditions.

The study population included women who were referred to the obstetric clinic in 2 hospitals in Lublin who were receiving antenatal care. Women willing to participate were asked to sign a consent forms.

A total of 84 patients in the third trimester of pregnancy were recruited: 41 with pregnancy-induced hypertension/pre-eclampsia (research group) and 43 healthy pregnant women (control group).

Inclusion criteria for the study included only singleton pregnant women, maternal age between 18 to 45 years, diagnosed with pregnancy-induced hypertension (PIH) or pre-eclampsia (PE) after 30 weeks of gestation, with blood pressure readings ≥140/90 mmHg pregnancies conceived naturally. All participants provided written informed consent to participate in the study.

Exclusion criteria included women who did not consent to participate in the study, as well as those with life-threatening conditions that would prevent study participation,

such as eclampsia, renal failure, or other severe comorbidities. Pregnancies resulting from in vitro fertilisation (IVF) were excluded.

The control group consisted of singleton healthy pregnant women with no history of hypertension, gestational diabetes, or other pregnancy complications. A standardised questionnaire was used to characterise patients including: gravidity, age, height, current weight, body mass index (BMI) and gestational age. Each patient in the third trimester of pregnancy had her blood drawn for analysis of vitamin D, its inactive form 25(OH)D, its active form $1,25(OH)_2D_3$, and calcium levels.

Measurements of serum 25(OH)D, $1,25(OH)_2D_3$, and calcium were performed using the LIAISON® XL analyzer (DiaSorin, Saluggia, Italy). The instrument was calibrated according to the manufacturer's instructions with certified reference materials. The lower limits of detection were 2 ng/mL for 25(OH)D and 5 pg/mL for $1,25(OH)_2D_3$. Internal quality control was performed daily at two concentration levels; the coefficients of variation were 6.2% for 25(OH)D and 7.8% for $1,25(OH)_2D_3$. The laboratory also participated in the national external quality assessment program (Polish Centre for Accreditation / national proficiency testing scheme), and all external quality control results were within acceptable limits.

The study was performed with the approval of the Bioethics Committee at the Medical University of Lublin, under Bioethics Committee Resolution No. KE-0254/248/12/2022 and Resolution No. KE-0254/95/05/2024. Determination of concentrations was performed by the hospital laboratories of the University Clinical Hospital No. 4 in Lublin and the Regional Specialist Hospital in Lublin.

Correlation analysis was conducted to assess relationships between serum vitamin D concentrations and other continuous variables, including ionised calcium and BMI. The selection of the correlation method—Pearson's product-moment correlation or Spearman's rank-order correlation—was based on variable distribution profiles.

All continuous demographic and biochemical variables were expressed as mean ± standard deviation (SD). Normality of distributions was assessed using the Shapiro–Wilk test and Q–Q plots, the equality of variances was assessed using Levene's test. For group comparisons, Student's t-test was applied to normally distributed data (reporting t and degrees of freedom), while the Mann–Whitney U test was used for non-parametric data (reporting U). Correlations were analyzed using Pearson's r for

normally distributed variables and Spearman's rho for non-normal variables, with all coefficients reported alongside 95% confidence intervals (CIs). To ensure transparency, all statistical test results, including those initially deemed non-significant, are reported with exact p-values and 95% CIs for mean differences. Where multiple biomarkers were tested, Benjamini–Hochberg false discovery rate (FDR) correction was applied, and both raw and adjusted p-values are presented. Independent risk factors for the development of hypertensive disorders of pregnancy were assessed using multivariable logistic regression. Given the number of biomarkers tested and the relatively small sample size, we did not apply formal corrections for multiple comparisons (e.g., FDR). Instead, the analyses are framed as exploratory, with emphasis placed on effect sizes and clinical plausibility rather than isolated p-values.

A post-hoc power calculation was performed for the two main comparisons (BMI and 25(OH)D levels between cases and controls) using a two-sample t-test ($\alpha = 0.05$, two-sided). The observed effect size (Cohen's d) for BMI was 1.21, yielding a post-hoc power of 99.9 %. For 25(OH)D, Cohen's d was 0.76, corresponding to a power of 74 %. These results indicate that the study was adequately powered to detect differences in BMI, and moderately powered for differences in 25(OH)D.

This study was observational in design, and its reporting adheres to the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines. This study followed the GAMER (Generative Artificial Intelligence in Medical Research) framework for transparent reporting of AI-assisted analyses. Generative AI tools were employed to support the structuring of statistical queries, validation of numerical consistency, table formatting, and biochemical unit conversions. Specifically, ChatGPT (OpenAI, GPT-5, September 2025 release) and Gemini Advanced (Google, 1.5 Pro, August 2025 update) were used in accordance with the GAMER guidelines. These tools served exclusively as analytical and editorial assistants and were not involved in primary data analysis or statistical modelling. All statistical calculations were independently conducted and re-verified using standard statistical software (R 4.3.3 and SPSS 29). Full responsibility for data integrity, accuracy of analyses, and clinical interpretations rests solely with the authors.

Ethical statement

This study was approved by the Bioethics Committee of the Medical University of Lublin (Resolutions No. KE-0254/248/12/2022 and KE-0254/95/05/2024). All participants provided written informed consent prior to inclusion in the study. The study was conducted in accordance with the principles of the Declaration of Helsinki.

Statistical analysis

Statistical analyses were performed using Statistica v13.3 (StatSoft Inc., Tulsa, OK, USA). Continuous variables are presented as mean ± standard deviation (SD). Data distribution was assessed using the Shapiro–Wilk test supported by inspection of Q–Q plots. For normally distributed variables, group comparisons were performed with the independent samples Student's t-test; for non-normally distributed variables, the Mann–Whitney U test was applied. Categorical variables were analysed using the χ^2 test or Fisher's exact test where appropriate. A two-tailed significance level of $\alpha = 0.05$ was adopted. Exact p-values and 95% confidence intervals are reported. Given the number of biomarkers analysed and the relatively small sample size, no formal correction for multiple testing was applied; instead, analyses are framed as exploratory, with interpretation focused on effect sizes and clinical plausibility.

RESULTS

The total number of participants in the study was 84 individuals who met inclusion criteria. The study included two cohorts: pregnancy-induced hypertension/pre-eclampsia (PIH/PE/research group) (n = 41) and normotensive controls (n = 43). All patients in both groups had singleton pregnancy conceived naturally. 3 participants in the PIH/PE group had confirmed pre-eclampsia; because of the small number, cases of PIH and PE were pooled for the main analysis. The demographic and clinical characteristics of participants in the research and control groups are summarised in Table 1.

Among PIH patients, 73.2% reported appropriate supplementation and 43.9% adhered to a proper diet. In comparison, 83.7% of healthy controls used supplementation and only 11.6% followed dietary recommendations. These findings suggest that while supplementation is relatively common in both groups, adherence to dietary guidelines is markedly lower among patients.

The mean maternal age was 31.5 ± 5.1 years in the PIH/PE group and 30.1 ± 5.3 years in controls (p=0.22, 95% CI for mean difference –0.88 to 3.97). A statistically significant difference was observed in the mean gestational age (GA) at the time of sample collection: the study group had a mean of 34.9 ± 3.3 weeks, whereas the control group had 37.2 ± 3.3 weeks (p=0.0022, 95% CI –3.70 to –0.74). This difference reflects the nature of the condition, as patients with PIH/PE often experience earlier delivery. The body mass index (BMI) was significantly higher in the study group (32.96 ± 5.18 kg/m²) compared with the control group (27.52 ± 3.77 kg/m², p<0.001, 95% CI 3.05 to 7.83). 12 women with gestational diabetes were present in the PIH/PE group and one in the control group.

Table 2 summarises the serum concentrations of the analysed biochemical parameters in both study groups. These include total calcium, ionised calcium, 25-hydroxyvitamin D (25(OH)D), and the active form of vitamin D_3 ($1,25(OH)_2D_3$). The values are compared between women diagnosed with pregnancy-induced hypertension or pre-eclampsia and normotensive controls. Where applicable, statistical significance was assessed using appropriate parametric or non-parametric methods.

The concentration of 25-hydroxyvitamin D (total vitamin D) showed a statistically significant difference between the groups. The mean concentration in the study group was 27.8 ± 10.2 ng/ml, placing this group in the insufficiency category (below 30 ng/ml, equivalent to 75 nmol/L). In the control group, the mean concentration was 35.7 ± 10.8 ng/ml. This difference was statistically significant (p=0.0118 mean difference –7.9 ng/mL (95% CI –14.0 to –1.8).

The mean concentrations of the active form of vitamin D did not differ significantly: 126.2 ± 40.3 pg/ml in the study group vs 136.4 ± 51.6 pg/ml in the control group (p=0.40, 95% CI –35.0 to 14.0). Both values are markedly elevated compared with the reference range for non-pregnant individuals (19.9–79.3 pg/ml), reflecting the physiological increase in renal and placental 1α-hydroxylase activity during the third trimester of pregnancy.

Neither total calcium (9.14 ± 0.42 vs 9.14 ± 0.39 mg/dl, p=0.98 (95% CI –0.19 to 0.18)) nor ionised calcium (1.214 ± 0.070 vs 1.205 ± 0.042 mmol/l, p=0.58 (95% CI –0.023 to 0.041)) showed statistically significant differences. This finding suggests

efficient calcium homeostatic mechanisms, which maintain its concentration within a narrow range, even at the expense of systemic reserves.

Scatter plots of BMI versus 25(OH)D and BMI versus ionised calcium are provided in Supplementary Figures S1–S2.

Correlation coefficients are:

- BMI and 25(OH)D: Spearman r = –0.04 (95% CI –0.32 to 0.25), p=0.76.
- BMI and ionised calcium: Pearson r = –0.11 (95% CI –0.39 to 0.19), p=0.47.
- 25(OH)D and $1,25(OH)_2D_3$: Spearman r = 0.16 (95% CI –0.13 to 0.42), p=0.29.

No correlations remained significant after Benjamini–Hochberg correction for multiple testing (FDR-adjusted p>0.1).

A multivariable logistic regression model was constructed to evaluate independent predictors of PIH/PE. After imputation of missing values, the final model included BMI, serum 25(OH)D, maternal age, gestational age, total calcium, ionized calcium, and gestational diabetes. In the adjusted analysis, BMI remained a significant risk factor (OR = 1.19 per 1 kg/m² increase; 95% CI 1.02–1.38; p = 0.023), while higher 25(OH)D concentrations were protective (OR = 0.92 per 1 ng/mL increase; 95% CI 0.85–0.997; p = 0.042). Gestational diabetes showed a non-significant trend towards increased risk, whereas maternal age, gestational age, total calcium, and ionized calcium did not reach statistical significance (all p > 0.2). Model calibration was acceptable (Hosmer–Lemeshow test, p = 0.62). Discriminative ability was fair, with an AUC of 0.78 (95% CI 0.67–0.88) and a Brier score of 0.19.

DISCUSSION

This study aimed to investigate the association between serum concentrations of total calcium, ionised calcium, 25-hydroxyvitamin D (25(OH)D), and the active form of vitamin D_3 ($1,25(OH)_2D_3$) and the occurrence of pregnancy-induced hypertension and pre-eclampsia. The main finding is that women with PIH/PE had significantly lower serum 25(OH)D concentrations and higher BMI compared with healthy controls, whereas calcium and $1,25(OH)_2D_3$ levels did not differ significantly. These findings support the hypothesis that disturbances in calcium–phosphate metabolism

and vitamin D insufficiency may contribute to the pathophysiology of hypertensive disorders in pregnancy [24, 25].

The mean serum 25(OH)D levels demonstrated a clear statistical and clinical difference. Patients in the research group had significantly lower levels of total vitamin D than healthy individuals, which may be associated with a higher risk of diseases resulting from vitamin D deficiency.

The active form of vitamin D ($1,25(OH)_2D_3$) regulates the transcription and function of genes associated with normal implantation, placental invasion, and angiogenesis. The differences in concentrations (126.2 vs. 136.4) identified in this study were too small, likely due to the limited sample size. Nonetheless, monitoring vitamin D levels in patients with pregnancy complications may be crucial due to its key role in calcium metabolism and bone health.

Because blood sampling occurred primarily in the third trimester after clinical diagnosis, our data should be interpreted as cross-sectional associations rather than evidence of causality or predictive utility. The observed lower 25(OH)D in women with PIH/PE is an association that warrants prospective, early-pregnancy investigation before any screening or preventive recommendations can be made.

Vitamin D, through the activation of the vitamin D receptor (VDR), modulates the expression of genes regulating cardiovascular system function as well as angiogenesis and trophoblast implantation processes. Deficiencies of this vitamin may lead to an endothelial dysfunction, increased oxidative stress, and activation of the renin–angiotensin–aldosterone system (RAA), which promotes increased vascular resistance and elevated blood pressure [26-28]. Vitamin D is proposed to affect blood pressure via the regulation of endothelial function [29].

The data demonstrated that total and ionised calcium levels were comparable between the groups. Maintaining ionised calcium within the clinical reference range, even in patients with PIH/PE, may result from effective physiological adaptation, such as increased production of $1,25(OH)_2D_3$ (as evidenced by elevated means in both groups) and mobilisation of bone reserves. However, this does not negate the hypothesis regarding calcium's role, as homeostasis may be preserved at the expense of bone health or hormonal mechanisms.

The study clearly indicates that the PIH/PE group is characterised by markedly higher obesity (BMI ≈ 33.0 kg/m²), consistent with the global risk profile for hypertensive disorders of pregnancy. Most patients do not follow dietary recommendations, resulting in excessive weight gain during pregnancy and subsequent obesity, which often becomes permanent [30]. Additionally, obese patients had a significantly higher risk of developing hypertension and other conditions such as diabetes. Being overweight, or obese, lowers the circulating levels of the active form of vitamin D in the blood due to its accumulation in adipose tissue, which may explain the lower levels of 25(OH)D observed in these patients [31–33]. The lack of a significant correlation between BMI and 25(OH)D ($r \approx -0.04$) in this cohort warrants critical discussion. Although a negative association is epidemiologically expected (due to sequestration in adipose tissue), this finding may reflect interventions (supplementation) later in pregnancy, which mitigated—but did not eliminate—the intergroup difference. Consequently, 25(OH)D concentrations in the third trimester are too variable and compensated to serve as a reliable risk predictor in this cross-sectional study.

Furthermore, in this observational study, the effect of vitamin D and calcium supplementation on serum concentrations of these substances was assessed in the context of pregnancy-induced hypertension and pre-eclampsia. Ideally, a healthy, balanced diet would be sufficient to maintain adequate levels of calcium and vitamin D during pregnancy. In this study, supplementation with either calcium or vitamin D was reported by 83.7% of women in the normotensive group, compared to only 73.2% in the group with gestational hypertension or pre-eclampsia. Although supplementation appeared more common among normotensive women, the timing, dosage, and type of supplementation were not standardised and therefore limit interpretability. This substantial difference may suggest a potential protective effect of supplementation, although causality cannot be established due to the observational nature of the study. Future research should focus on the potential preventive role of early and adequate calcium and vitamin D supplementation, particularly in women with elevated BMI, who may be at higher risk of metabolic disturbances and hypertensive complications.

Our observations, confirming insufficient 25(OH)D status and high obesity in the PIH/PE group, support the recommendation for individualized supplementation.

Patients with obesity (BMI ≈ 33 kg/m²) may require higher doses, as suggested by previous trials, but this needs confirmation in early pregnancy RCTs [27].

The timing of the initiation of vitamin D supplementation plays a crucial role in preventing the onset of hypertension. Deficiencies occurring in the first trimester of pregnancy may be especially significant in the pathogenesis of pre-eclampsia, due to key processes taking place during placental development and spiral artery remodeling [34-36]. WHO recommends daily calcium supplementation of 1.5–2.0 g for pregnant women in populations with low dietary calcium intake to reduce pre-eclampsia risk [37].

Strengths include the use of standardised laboratory assays, multivariable adjustment for confounders (BMI, gestational age, diabetes), and transparent reporting of effect sizes, CIs, and corrected p-values.

This study has several limitations. Testing vitamin D and calcium levels in the third trimester may not reflect the true risk of pre-eclampsia because, as mentioned earlier, it is the first trimester that is crucial in this regard. This is particularly problematic when trying to predict the risk of pre-eclampsia in the third trimester of pregnancy. This is when current serum levels appear normal - probably due to the patient starting vitamin D and calcium supplementation in the second trimester of pregnancy. Samples were collected after diagnosis; thus, associations cannot be interpreted as predictive or causal. Seasonality and dietary calcium intake were not measured. Checking bone mineralisation levels in the third trimester using radiofrequency echographic multispectrometry (REMS) or the quantitative ultrasound (QUS) may give some indication of the rate of vitamin D levels in the first trimester.

Findings support the hypothesis that vitamin D insufficiency and obesity contribute to the pathophysiology of PIH/PE. However, given the observational design, our data should be interpreted as exploratory. Early pregnancy trials are needed to evaluate vitamin D and calcium supplementation as preventive strategies. Current guidelines recommending adequate supplementation (e.g. 600–1000 mg calcium, 600–2000 IU vitamin D daily, higher in obese women) remain the evidence-based standard.

Finally, monitoring 25-hydroxyvitamin D levels in pregnant women may help identify those at increased risk of gestational hypertension and pre-eclampsia. Further

research involving larger cohorts and long-term follow-up is needed to clarify the preventive role of vitamin D and calcium supplementation.

CONCLUSION

It should be emphasized that due to the cross-sectional design of this study, the findings are associative and do not permit causal inference. Women with hypertensive disorders of pregnancy demonstrated higher BMI and significantly lower 25(OH)D concentrations compared with normotensive controls, whereas calcium and $1,25(OH)_2D_3$ levels did not differ. These cross-sectional findings reinforce the association between metabolic disturbances and PIH/PE, but cannot establish causality. Larger, prospective studies are warranted to clarify whether early vitamin D and calcium optimisation can reduce the risk of hypertensive complications.

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Data availability: The datasets generated and analysed during the current study are not publicly available due to patient confidentiality and ethical restrictions, but are available from the corresponding author on reasonable request and with appropriate institutional approvals.

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TABLES AND FIGURES WITH LEGENDS

Table 1. Participant characteristics

Characteristic	Research Group (*n*=41)	Control Group (*n*=43)
Mean age (Years)	31.5	30.1
Mean BMI (kg/m2)	32.96±5.18	27.52±3.77
Mean gestational age (weeks)	34.9±3.3	37.2±3.3
Pregnancy count - % distribution	I-51.22%, II-17.07%, III-9.76%, IV-9.76%, V-9.76%, VII-2.43%	I-55.81%, II-32.56, III-9.3%, V-2.33%
Vitamin D3 supplementation	73.2%	83.7%
Diet modification reported	43.9%	11.6%
Most frequent comorbidities	GDM 28%, Hypothyroidism 26%, Pre-eclampsia 7%.	N/a

Note: Values are presented as means ± standard deviations. Research Group: Patients with diagnosed hypertension. Control Group: Patients without recorded comorbidities. Supplementation: Includes any prenatal vitamins or mineral intake. Pregnancy count indicates the number of pregnancies a participant has experienced, with percentages shown for each occurrence (e.g., first, second, third). Diet Modification: Vegetarian, gluten-free, diabetic, low glycaemic index, or light diets. Comorbidities: Only non-hypertensive conditions reported. Abbreviations: BMI: Body mass index; D_3: Vitamin D3 (cholecalciferol); GDM: Gestational diabetes mellitus; N/A: Not applicable.

Table 2. Serum parameter concentrations

Parameter	Research Group	Control Group	*p* value	Interpretation
Total calcium (mg/dl)	9.14±0.42	9.14±0.39	0.98	No significant difference
Ionised calcium (mmol/L)	1.214±0.070	1.205±0.042	0.58	No significant difference
Total 25(OH)D (ng/mL)	27.8±10.2	35.7±10.8	0.0118	**Statistically and clinically significant difference**
Vitamin D3 ($1,25(OH)_2D_3$) (pg/mL)	126.2±40.3	136.4±51.6	0.4	No significant difference

Note: Values of total calcium, ionized calcium, vitamin D_3 (1,25(OH)2D_3), and total vitamin D (25(OH)D) are presented as means ± standard deviations for patients with pregnancy-induced hypertension (PIH) and healthy controls. Results of Student's t-test are presented along with significance levels. In healthy persons, levels equal 25 to 40 ng/mL (62.4 to 99.8 nmol/L) for 25(OH) D_3 and 19,9 -79,3 pg/mL for 1,25(OH)2D_3. The normal serum total calcium concentration in an adult is 8.6−10.2 mg/dl, ionised calcium 1.15-1.32 mmol/l.

Table 3. Multivariable logistic regression analysis for predictors of pregnancy-induced hypertension

Variable	OR	95% CI	*p* value
BMI (kg/m²)	1.19	1.02–1.38	**0.023**
25(OH)D (ng/mL)	0.92	0.85–1.00	**0.042**
Gestational week	0.90	0.78–1.03	0.118

Note: Results of multivariable logistic regression identifying independent predictors of pregnancy-induced hypertension (including pre-eclampsia). The model included maternal BMI, serum 25(OH)D concentration, gestational week. Statistically significant predictors were higher BMI, lower 25(OH)D concentration. Odds ratios (OR) are presented with 95% confidence intervals (CI) and p-values. Abbreviation: BMI: Body mass index.

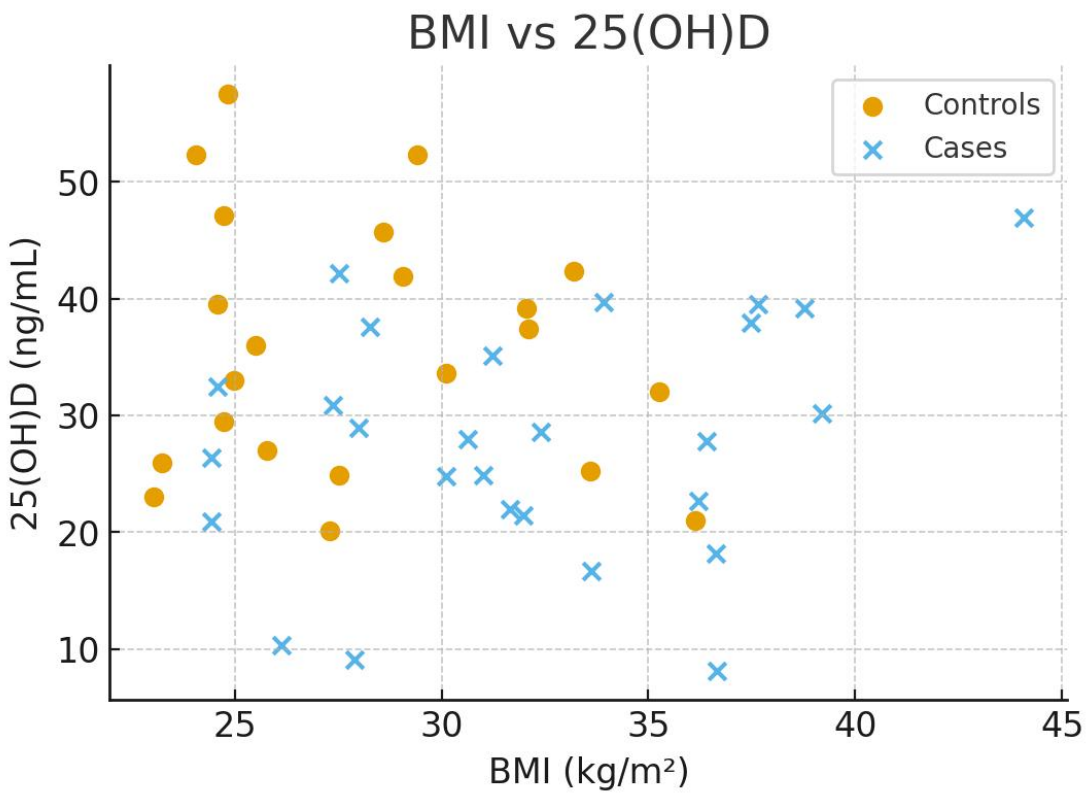

Figure 1. Scatter plot showing the relationship between maternal body mass index (BMI, kg/m²) and serum 25-hydroxyvitamin D [25(OH)D] concentration (ng/mL). Each point represents one participant; the solid line indicates the regression fit with 95% confidence interval (shaded area). Abbreviation: BMI: Body mass index.

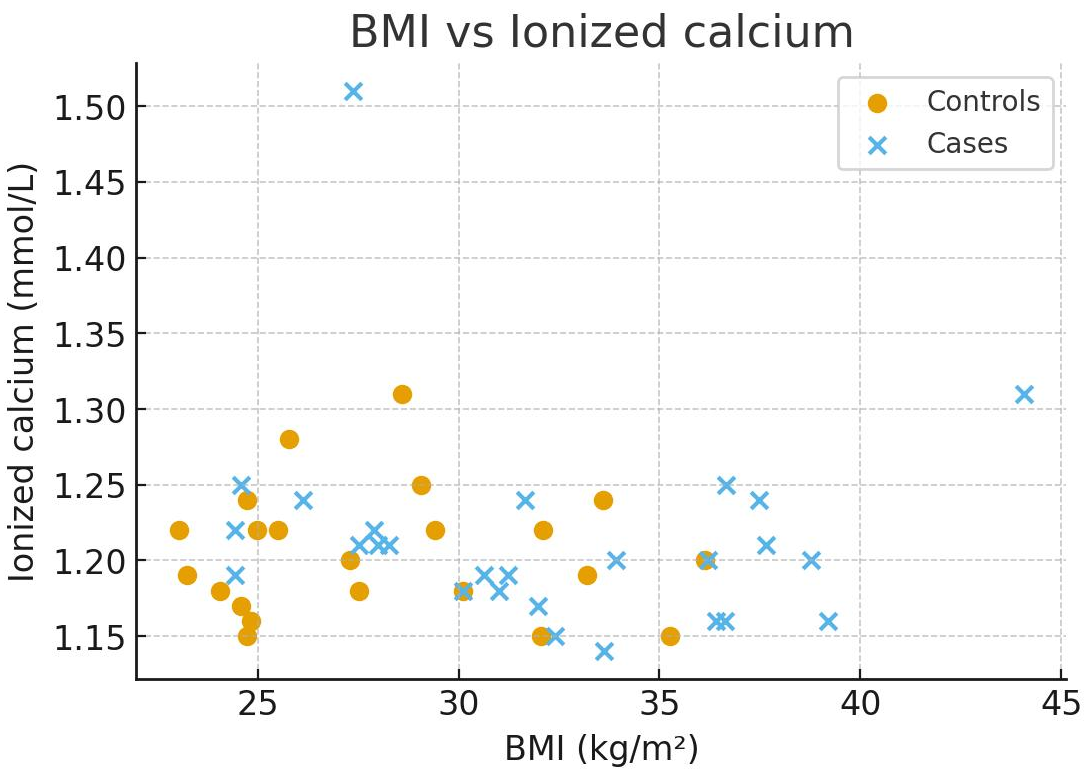

Figure 2. Scatter plot showing the relationship between maternal body mass index (BMI, kg/m²) and serum ionised calcium concentration (mmol/L). Each point represents one participant; the solid line indicates the regression fit with 95% confidence interval (shaded area). Abbreviation: BMI: Body mass index.