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FIBROBLAST GROWTH FACTOR 23, CALCIUM AND PHOSPHATE SERUM LEVELS IN EXPERIMENTAL PREECLAMPSIA: IMPACT OF VITAMIN D_3 STATUS

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Preeclampsia (PE) is a major cause of maternal and perinatal morbidity, with its pathogenesis involving, in particular, impaired mineral homeostasis. Recent evidence suggest that fibroblast growth factor 23 (FGF23), a key regulator of phosphate balance and vitamin D_3 metabolism, may contribute to pregnancy complications, however, its role in PE remains poorly understood. This study aimed to evaluate serum FGF23 level and its relationship with vitamin D_3 and calcium–phosphate balance at preeclampsia development in animal experimental model. Thirty-five Wistar female rats were divided into three groups: controls on a standard diet; vitamin D_3-deficient rats; vitamin D_3-deficient rats supplemented with cholecalciferol. Within each group, PE was induced by Nω-nitro-L-arginine methyl ester administration. Serum concentrations of 25(OH)D_3, FGF23, parathyroid hormone (PTH), total calcium, inorganic phosphate, and alkaline phosphatase (ALP) activity were determined by ELISA and biochemical assays. It was shown that vitamin D_3 deficiency was accompanied by hypocalcemia, hypophosphatemia, elevated FGF23 and increased ALP activity in the serum. Supplementation with vitamin D_3 increased 25(OH)D_3, markedly reduced FGF23 levels and normalized mineral parameters. Induction of PE caused significant disturbances in calcium–phosphate status, hypertension, and 100% mortality of vitamin D_3-deficient animals. In the presence of preeclampsia vitamin D_3 efficacy was limited. In PE group, despite of vitamin D_3 supplementation, serum FGF23 was markedly elevated, indicating impaired vitamin D_3 metabolism. Our findings demonstrate that vitamin D_3 deficiency amplifies PE severity through disruption of mineral homeostasis and FGF23 dependent signaling.

K e y w o r d s: 25(OH)D_3, vitamin D_3 deficiency, L-NAME, preeclampsia, calcium–phosphate metabolism.

Preeclampsia (PE) remains one of the leading causes of maternal and perinatal morbidity and mortality worldwide, representing a major medical and social challenge in modern obstetrics [1, 2]. This multifactorial syndrome, typically manifesting after the 20th week of gestation, is characterized by newly diagnosed arterial hypertension in combination with proteinuria and is accompanied by a wide spectrum of systemic disorders. The pathogenesis of preeclampsia involves complex interactions among defective vascular remodeling, endothelial dysfunction, systemic inflammation, and immune response imbalance [3, 4]. Despite substantial progress in understanding risk factors and molecular mechanisms underlying this condition, highly specific and sensitive biomarkers for its early detection and prediction, as well as effective preventive strategies with proven clinical benefit, are still lacking.

In recent years, there has been growing interest in the role of hormonal regulators of calcium–phosphate metabolism in the pathogenesis of pregnancy complications, particularly preeclampsia [5, 6]. Of special interest is fibroblast growth factor 23 (FGF23), a hormone-like protein predominantly secreted by osteocytes and osteoblasts, which plays a key role in maintaining phosphate homeostasis and regulating vitamin D$_3$ metabolism [7-9]. At the level

of renal proximal tubules, FGF23 reduces phosphate reabsorption by suppressing the sodium–phosphate co-transporters NaPi-IIa and NaPi-IIc, while simultaneously inhibiting the activity of 1α-hydroxylase 25-hydroxyvitamin D (CYP27B1) – the enzyme responsible for the synthesis of the biologically active form of vitamin D_3 (calcitriol, $1\alpha,25(OH)_2D_3$). In parallel, it induces the expression of 24-hydroxylase (CYP24A1), which catalyzes the degradation of vitamin D_3, thereby decreasing its bioavailability. These effects are mediated through the activation of intracellular signaling cascades (mainly ERK1/2) following the interaction of FGF23 with its receptor FGFR1 in the presence of the co-receptor α-Klotho.

Experimental and clinical evidence indicates that excessive FGF23 activity leads to a significant reduction in $1\alpha,25(OH)_2D_3$ concentrations, both through the inhibition of its synthesis and through enhanced catabolic degradation [10, 11]. Importantly, the impact of FGF23 is not confined to renal tissue but also extends to extra-renal sources of active vitamin D_3 production, which is of fundamental significance under conditions associated with its deficiency.

Vitamin D_3, represented both by its circulating form 25-hydroxyvitamin D_3 ($25(OH)D_3$) and its biologically active metabolite $1\alpha,25(OH)_2D_3$, performs a range of critically important functions during pregnancy: it regulates immunological tolerance, supports endothelial function, promotes placental vascularization, and facilitates trophoblast differentiation [12-15]. Maternal deficiency of $25(OH)D_3$ has been associated with an increased risk of preeclampsia, intrauterine growth restriction, and preterm birth. Although meta-analyses and systematic reviews of clinical and experimental studies indicate a potential protective effect of adequate vitamin D_3 bioavailability, the results of interventional trials remain inconsistent due to variability in supplementation dosage, timing of administration, and population characteristics [16-18].

Data regarding alterations in FGF23 levels during pregnancy and in preeclampsia remain inconsistent. Some studies report its serological elevation in affected mothers, while others demonstrate a decrease [19]. These discrepancies may be attributed to differences in preeclampsia phenotypes (early- vs. late-onset and severity), timing of sample collection, and methodological aspects of measurement. Recent clinical studies show correlations between FGF23 concentrations and biomarkers of placental dysfunction, suggesting its potential involvement in the pathogenesis of preeclampsia, although a comprehensive understanding of this association has yet to be fully established [20]. Experimental models indicate a possible influence of FGF23 on the expression of placental genes involved in vitamin D_3 metabolism (particularly Cyp24a1), as well as its capacity to indirectly modulate angiogenesis. At present, it remains relevant to elucidate the relationship between FGF23-mediated signaling, vitamin D_3 metabolism, and mineral homeostasis in both normal and pathological pregnancy.

A relevant scientific challenge is to elucidate the role of fibroblast growth factor 23 in the regulation of vitamin D_3 metabolism and its impact on pregnancy under both physiological and complicated conditions. In particular, the relationship between changes in FGF23 levels, disturbances in calcium–phosphate homeostasis, and the development of PE remains insufficiently understood. In this context, the objective of our study was to assess FGF23 levels during pregnancy and determine their association with preeclampsia in an experimental model.

Materials and Methods

Animals and general experimental design. The experimental cohort consisted of 35 eight-week-old female Wistar rats with a mean body weight of 174 ± 12 g. All animals were housed under standard vivarium conditions (temperature 22 ± 2°C, relative humidity 55–60%, 12-h light/dark cycle) with unrestricted access to food and water.

The animals were allocated into three experimental groups (Fig. 1). Group I (n = 10) – control females fed a standard vivarium diet without vitamin D_3 restrictions. Group II (n = 12) – females fed a rachitogenic diet (wheat flour – 89.5%, $CaCO_3$ – 3%, NaCl – 2%, dried brewer's yeast – 0.01%, vitamin A – 0.01%, vitamin E – 0.01%, water-soluble vitamins (Complevit) – 0.02%) completely lacking vitamin D_3 for 60 days before mating to establish a vitamin D_3-deficient status. Group III (n = 13) – females subjected to the same vitamin D_3-deficient diet for 60 days, followed by transition to a regular laboratory diet supplemented with oral vitamin D_3. Cholecalciferol (Sigma, USA) was administered in sunflower oil via gavage at a dose of 1000 IU/kg body weight (0.2 ml oil solution) daily for two weeks prior to mating.

Ethical statement. All experimental procedures were conducted following established bioethi-

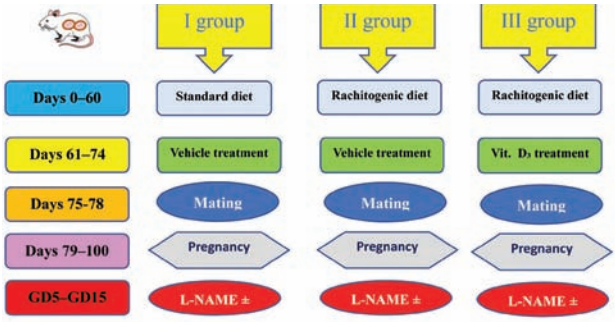

Fig. 1. Experimental timeline for rat groups

cal principles and international regulations for the use of laboratory animals, including EU Directive 2010/63/EU, the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986), and the Ukrainian Guidelines for Bioethical Assessment of Preclinical and Other Scientific Research on Animals (Kyiv, 2006). The study was approved by the Bioethics Committee of Bogomolets National Medical University (Protocol No. 123, November 4, 2024).

Induction of vitamin D_3 deficiency. Groups II and III were fed a vitamin D_3–deficient rachitogenic diet for 60 days prior to mating to induce deficiency [16]. Blood samples were collected from the tail vein to assess 25-hydroxyvitamin D_3 ($25(OH)D_3$) levels using a General 25-Hydroxyvitamin D_3 ELISA kit (UNDL00047, Assay Genie, Ireland), and concentrations were calculated with Gain Data® (Arigo's ELISA Calculator).

Mating and pregnancy monitoring. After confirming vitamin D_3 deficiency, the females were mated with fertile males at a male-to-female ratio of 2:1. The presence of spermatozoa in vaginal smears the next morning was used to define gestational day 1 (GD1).

Induction of preeclampsia. To induce L-NAME–mediated preeclampsia (PE), each experimental group was divided into subgroups A and B. Animals in subgroup B received subcutaneous injections of L-NAME (Nω-nitro-L-arginine methyl ester) at a dose of 100 mg/kg body weight on GD5 and GD7 [21]. From GD5 to GD15, the animals were further administered L-NAME daily in drinking water at a dose of 40 mg/kg body weight. Rats in subgroup A continued on the respective diet and received two subcutaneous injections of physiological saline (vehicle).

Blood pressure measurement. Blood pressure was measured using the CODA® Monitor (Kent Scientific, USA), a non-invasive tail-cuff system based on volume pressure recording (VPR). Measurements were taken on GD1 (baseline) and GD15 (following 10 days of L-NAME treatment). Rats were placed in restrainers on a thermostatically controlled platform (37–38°C) to ensure stable blood flow, and 3–5 readings were obtained within 5–7 minutes to minimize variability.

Determination of calcium–phosphate metabolism and related biomarkers. The serum content of FGF23 in pregnant rats was measured using an enzyme-linked immunosorbent assay (ELISA) with the Rat FGF23 (Fibroblast Growth Factor 23) ELISA kit (RTFI00086; AssayGenie, Ireland) according to the manufacturer's protocol.

Parathyroid hormone (PTH) levels were determined by ELISA using the Rat Pth (Parathyroid Hormone) ELISA kit (RTFI00259; AssayGenie, Ireland) following the manufacturer's instructions. The concentrations of FGF23 and PTH in the analyzed samples were calculated using GainData® (arigo's ELISA Calculator).

Serum calcium levels were assessed with a commercial bio-test kit (Lachema, Czech Republic), using a standard solution of 25 mmol/l $CaCO_3$ dissolved in 1.7% HCl. The method is based on the ability of calcium to form, in an alkaline medium, a red-colored complex with glyoxal-bis-2(oxyanil), which was quantified spectrophotometrically at $\lambda = 540$ nm.

The concentration of inorganic phosphate (Pi) in serum was determined after protein precipitation with 12% trichloroacetic acid (TCA) using the Dyce method. Protein-free extracts (0.5 ml) were incubated at 37°C for 10 min with ascorbic acid (260 mM) and ammonium molybdate (7 mM). After incubation, the samples were placed on ice for 10 min, and optical density was measured spectrophotometrically at $\lambda = 640$ nm.

The activity of alkaline phosphatase (alkaline phosphomonoesterase, orthophosphoric acid monoester hydrolase) in serum was determined by measuring the formation of 4-nitrophenol resulting from the hydrolysis of the substrate 4-nitrophenylphosphate. The reaction was stopped by adding 30 mM Trilon B solution in 1 M NaOH. The optical density of the samples was measured spectrophotometrically at $\lambda = 410$ nm. The activity of total alkaline phosphatase was calculated using the following formula:

$X = \varepsilon(sample)/\varepsilon(standard) \times K$,

where X is the number of micromoles of 4-nitrophenol released by the enzyme per liter of serum per minute at 37°C; 0.096 – mmol of 4-nitrophenylphosphate in 0.04 ml of standard solution; 0.04 – serum volume (ml); 15 – incubation time (min).

The activity of the bone-specific alkaline phosphatase isoform was determined as the difference between the total alkaline phosphatase activity and the thermostable alkaline phosphatase activity, after heating the samples in a water bath at 56–57°C.

Statistical analysis. Data were analyzed using conventional methods of variation statistics, with the calculation of the mean (M) and standard error of the mean (±m). Statistical significance of differences between groups was assessed using one-way ANOVA followed by Bonferroni post hoc correction. Differences were considered significant at $P < 0.05$. Statistical processing was performed using OriginLab 8.5 software.

Results and Discussion

Serum 25-hydroxyvitamin D_3, the primary hydroxylated metabolite of cholecalciferol, serves as a reliable marker of systemic vitamin D_3 bioavailability due to its prolonged half-life (2–3 weeks), reflecting both endogenous synthesis and dietary or supplemental intake. In the present study, we observed that serum 25(OH)D_3 concentrations in pregnant female rats were significantly modulated by dietary regimen (standard versus rachitogenic), exogenous vitamin D_3 supplementation, and the presence of experimental preeclampsia, highlighting the combined influence of nutritional status and pathological conditions on vitamin D_3 homeostasis during gestation.

As shown in Fig. 2, in animals maintained on a vitamin D_3-deficient diet (Group IIA), the mean serum 25(OH)D_3 level was 16.5 ± 1.2 ng/ml, which was nearly threefold lower ($P < 0.001$) compared with the control Group IA (49.6 ± 2.4 ng/ml) and corresponds to the development of severe vitamin D_3 deficiency (Holick, 2011). In Group IIB (vitamin D_3 deficiency with preeclampsia), all animals died, indicating high mortality associated with the combination of profound vitamin D_3 deficiency and preeclampsia. Consequently, biochemical data for this group were not obtained.

Administration of vitamin D_3 to rats (Group IIIA) resulted in a significant increase in 25(OH)D_3 levels to 34.6 ± 2.1 ng/ml ($P < 0.01$ vs. IA); however, in Group IIIB (with preeclampsia), 25(OH)D_3 decreased to 30.7 ± 1.2 ng/ml ($P < 0.05$ vs. IIIA), indicating partial attenuation of vitamin D_3 supplementation efficacy in the presence of preeclampsia. Intact animals (Group IA) exhibited the highest serum 25(OH)D_3 levels (49.6 ± 2.4 ng/ml), whereas the

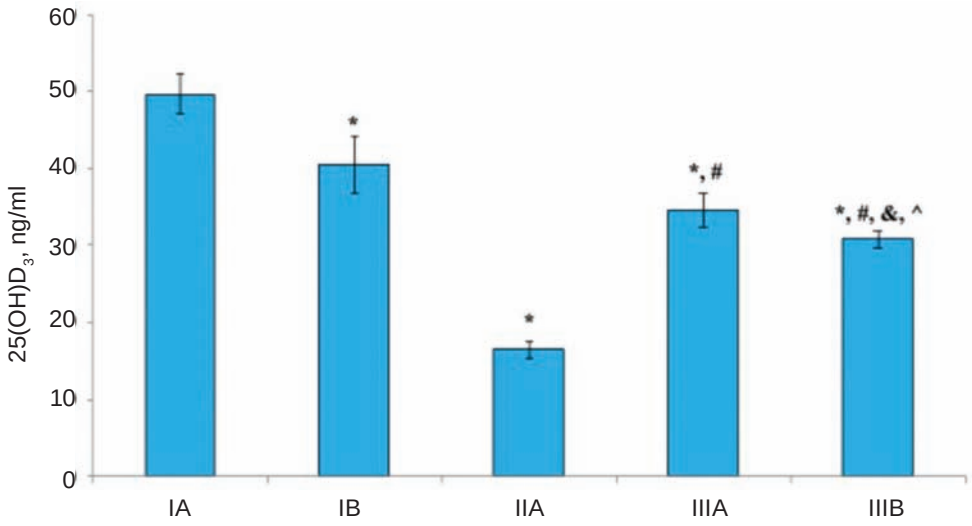

*Fig. 2. Serum 25(OH)D_3 levels in the experimental groups (M ± m): IA – control group without preeclampsia, IB – control group + preeclampsia, IIA – vitamin D_3-deficiency, without preeclampsia, IIIA – vitamin D_3-deficiency + vitamin D_3, without preeclampsia, IIIB – vitamin D_3-deficiency + vitamin D_3 + preeclampsia. *Statistically significant difference compared to Group IA; #statistically significant difference compared to Group IIA; &statistically significant difference compared to Group IIIA; ^statistically significant difference compared to Group IB, $P < 0.05$*

development of preeclampsia in this group (Group IB) was associated with a reduction to 40.4 ± 3.6 ng/ml ($P < 0.01$ vs. control).

One of the key diagnostic criteria for preeclampsia in the studied groups was the presence of hemodynamic alterations, specifically hypertension, as presented in Fig. 3.

In the group of animals with vitamin D_3 deficiency (IIA), on gestational day 15 (GD15), mean systolic/diastolic blood pressure values were 132.00 ± 2.31/88.90 ± 2.04 mmHg, which was significantly higher ($P < 0.01$) compared with control rats in Group IA (110.20 ± 4.56/69.60 ± 4.01 mmHg). In Group IIB (vitamin D_3 deficiency + preeclampsia), all animals died prior to blood pressure measurement, confirming the severe progression of preeclampsia induced by L-NAME in the context of vitamin D_3 deficiency.

Vitamin D_3 supplementation in Group IIIA (without preeclampsia) was associated with a reduction in blood pressure to 118.50 ± 4.27/77.00 ± 1.69 mmHg ($P < 0.05$ vs. IIA), indicating a beneficial effect of vitamin D_3 correction on vascular tone and hemodynamics. However, in the presence of preeclampsia with vitamin D_3 supplementation (Group IIIB), blood pressure increased to 174.70 ± 5.11/118.00 ± 5.67 mmHg ($P < 0.001$ vs. IIIA), which exceeded even the hypertension observed in Group IIA. In the control Group IA, blood pressure remained within the physiological range (110.20 ± 4.56/69.60 ± 4.01 mmHg), whereas the development of preeclampsia in Group IB was accompanied by an increase to 163.80 ± 2.76/105.20 ± 3.57 mmHg ($P < 0.001$ vs. intact controls). These findings indicate that the combination of vitamin D_3 deficiency and preeclampsia leads to more pronounced hemodynamic disturbances than any other experimental condition studied in this model.

Considering the direct involvement of the hormonally active form of vitamin D_3 in the formation of the inorganic bone matrix and in the regulation of calcium–phosphate metabolism, we further analyzed changes in blood mineral components. The observed alterations were generally consistent with the vitamin D_3 status of pregnant rats under normal conditions, as well as during the induction of nutritional vitamin D_3 deficiency and L-NAME-induced preeclampsia (Table).

In Group IIA, total serum calcium was decreased to 1.87 ± 0.02 μmol/min·l compared with 2.30 ± 0.04 μmol/min·l in intact controls ($P < 0.001$), reflecting the development of classical hypocalcemia under conditions of low vitamin D_3 status. In Groups IIIA and IIIB, calcium levels increased to 1.92 ± 0.02 and 1.94 ± 0.02 μmol/min·l, respectively, correlating with elevated serum 25(OH)D_3 concentrations and indicating partial restoration of calcium homeostasis following vitamin D_3 supplementation. In control animals with induced preeclampsia (Group IB), serum calcium decreased significantly to 2.10 ± 0.01 μmol/

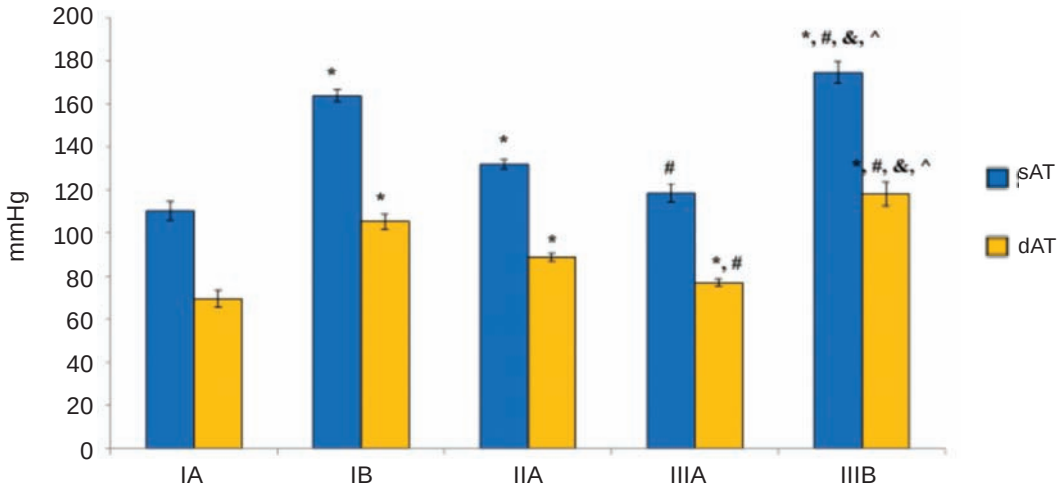

*Fig. 3. Blood pressure characteristics in the experimental groups (M ± m): IA – control group without preeclampsia, IB – control group + preeclampsia, IIA – vitamin D_3-deficiency, without preeclampsia, IIIA – vitamin D_3-deficiency + vitamin D_3, without preeclampsia, IIIB – vitamin D_3-deficiency + vitamin D_3 + preeclampsia. *Statistically significant difference vs. Group IA; #statistically significant difference vs. Group IIA; &statistically significant difference vs. Group IIIA; ^statistically significant difference vs. Group IB, $P < 0.05$*

T a b l e . Calcium–phosphate metabolism parameters

Indicator	Control (Group I), *n* = 10		D_3 hypovitaminosis (Group II), *n* = 6	D_3 hypovitaminosis + vitamin D_3 (Group III), *n* = 13	
	IA (without PE), *n* = 5	IB (PE), *n* = 5	IIA (without PE), *n* = 6	IIIA (without PE), *n* = 7	IIIB (PE), *n* = 6
Total calcium, μmol/min·l	2.30 ± 0.04	2.10 ± 0.01^	1.87 ± 0.02*	1.92 ± 0.02$^{\#}$	1.94 ± 0.02$^{\&}$
Inorganic phosphate, μmol/min·l	2.15 ± 0.03	2.29 ± 0.02^	1.80 ± 0.03*	2.02 ± 0.07$^{\#}$	2.15 ± 0.04$^{\&}$
Total alkaline phosphatase, μmol/min·l	207.6 ± 1.5	276.90 ± 3.29^	325.6 ± 2.4*	244.40 ± 3.84$^{\#}$	347.70 ± 7.01$^{\&}$
Bone isoenzyme of alkaline phosphatase, μmol/min·l	196.30 ± 3.03	259.5 ± 5.6^	302.80 ± 4.05*	236.20 ±3.97$^{\#}$	327.40 ± 7.98$^{\&}$

Note. Group IIB(PE) – all animals died demonstrating extremely high mortality associated with vitamin D_3 deficiency and preeclampsia. *Statistically significant difference vs. IA; $^{\#}$statistically significant difference vs. IIA; $^{\&}$statistically significant difference vs. IIIA; ^statistically significant difference vs. IB; $P < 0.05$

min·l compared with rats not receiving L-NAME ($P < 0.05$).

Serum inorganic phosphate concentration in vitamin D_3-deficient animals (Group IIA) decreased to 1.80 ± 0.03 μmol/min·l compared with 2.15 ± 0.03 μmol/min·l in intact controls ($P < 0.05$; Table). Correction of vitamin D_3 deficiency with cholecalciferol in Group IIIA resulted in the Pi elevation to 2.02 ± 0.07 μmol/min·l, while in the context of preeclampsia induction (Group IIIB), levels rose to 2.15 ± 0.04 μmol/min·l ($P < 0.05$). In control animals, the development of preeclampsia (Group IB) was associated with a further increase in Pi to 2.29 ± 0.02 μmol/min·l ($P < 0.05$ vs. IA), which likely reflects the adverse effects of preeclampsia and renal dysfunction on phosphate excretion.

In the control group (I), the mean total alkaline phosphatase (ALP) activity was 207.6 ± 1.5 μmol/min·l, whereas in animals with vitamin D_3 deficiency (II), this parameter increased to 276.9 ± 3.29 μmol/min·l. The highest values were observed in the vitamin D_3-supplemented group (III) – 325.6 ± 2.40 μmol/min·l ($P < 0.05$ vs. control), which may reflect compensatory enhancement of mineralization processes in response to restored vitamin D status. Analysis of subgroups differing by preeclampsia status showed that in animals without PE (IA, IB, IIA, IIIA), total ALP activity ranged from 244.4 to 347.7 μmol/min·l, whereas in the presence of PE (IIIB) an additional increase to 347.7 ± 7.01 μmol/min·l was observed.

Bone-specific ALP isoenzyme demonstrated a similar pattern: 196.3 ± 3.03 μmol/min·l in controls, 259.5 ± 5.60 μmol/min·l in the vitamin D_3-deficient group, and 302.8 ± 4.05 μmol/min·l in the vitamin D_3-supplemented group ($P < 0.05$ vs. control). In the IIIB subgroup (PE), this parameter reached 327.4 ± 7.98 μmol/min·l, exceeding values in animals without PE, indicating a possible contribution of pathological pregnancy to bone tissue remodeling and osteoblast activation.

Increased activity of total alkaline phosphatase, particularly its bone isoform, may reflect disturbances in the deposition of calcium and phosphate in bone tissue and/or enhanced bone resorption as a key mechanism for mobilizing mineral components to maintain systemic calcium–phosphate homeostasis.

The classical response to hypocalcemia and hypophosphatemia associated with vitamin D_3 deficiency is the development of secondary hyperparathyroidism [22]. Analysis of serum parathyroid hormone levels demonstrated elevations above reference values for young non-pregnant female rats in all experimental groups. According to the literature, normal physiological PTH levels in non-pregnant females range from 15 to 65 pg/ml [23].

In vitamin D_3-deficient animals (Group IIA), PTH concentration reached 266.9 ± 8.7 pg/ml compared with 326.5 ± 13.0 pg/ml in the intact control group ($P < 0.05$; Fig. 4). Vitamin D_3 supplementation in Group IIIA resulted in an increase of PTH to 310.7 ± 9.8 pg/ml ($P < 0.05$ vs. IIA), likely reflecting activation of calcium metabolism and enhanced bone turnover. In Group IIIB, preeclampsia induction led to a decrease in PTH to 266.9 ± 15.6 pg/ml ($P < 0.05$ vs. IIIA), possibly highlighting significant renal impairment and suppression of PTH secretion.

In control rats (Group IA), PTH levels were the highest among all groups (326.5 ± 13.0 pg/ml), whereas preeclampsia induction (Group IB) reduced PTH to 272.7 ± 9.9 pg/ml ($P < 0.05$), confirming the probable inhibitory effect of L-NAME-induced preeclampsia on hormone secretion under the conditions of this experiment.

Fibroblast growth factor 23 (FGF23) is an important regulator of phosphate metabolism and placental function, with serum levels exhibiting significant and highly contrasting intergroup changes (Fig. 5).

The highest concentrations of this growth factor were observed in vitamin D_3-deficient animals (Group IIA), exceeding more than twice those of the intact control group (473.2 ± 10.59 vs. 208.2 ± 11.16 pg/ml, $P < 0.001$). This may reflect activation of the mechanisms for enhanced renal phosphate excretion and the development of phosphaturia under conditions of vitamin D_3 deficiency. Partial correction of vitamin D_3 deficiency by cholecalciferol supplementation in Group IIIA elicited a marked decrease in FGF23 levels to 167.6 ± 5.57 pg/ml compared with the D_3-deficient group ($P < 0.001$). Upon induction of preeclampsia (Group IIIB), FGF23 levels increased to 386.5 ± 28.74 pg/ml compared with Group IIIA ($P < 0.01$), likely reflecting pronounced preeclampsia-associated renal dysfunction. In control rats, FGF23 levels remained unchanged during preeclampsia modeling (214.6 ± 14.99 pg/ml) compared with intact animals (208.2 ± 11.16 pg/ml).

The results of this study in an experimental rat pregnancy model confirm the pivotal role of vitamin D_3 in the hormonal regulation of calcium–phosphate metabolism under both physiological conditions and during the development of preeclampsia. The observed reduction in serum 25(OH)D_3 levels to nearly 16 ng/ml in vitamin D_3-deficient animals indicates the development of severe vitamin D_3 deficiency according to current classifications of vitamin D_3 status [24]. Previous studies have shown that low 25(OH)D_3 levels are associated with an increased risk of preeclampsia in pregnant women [25]. The 100% mortality observed in animals with experimentally induced preeclampsia on the background of severe vitamin D_3 deficiency highlights the compounded negative impact of these two factors (D_3 deficiency + PE), consistent with other experimental studies [26].

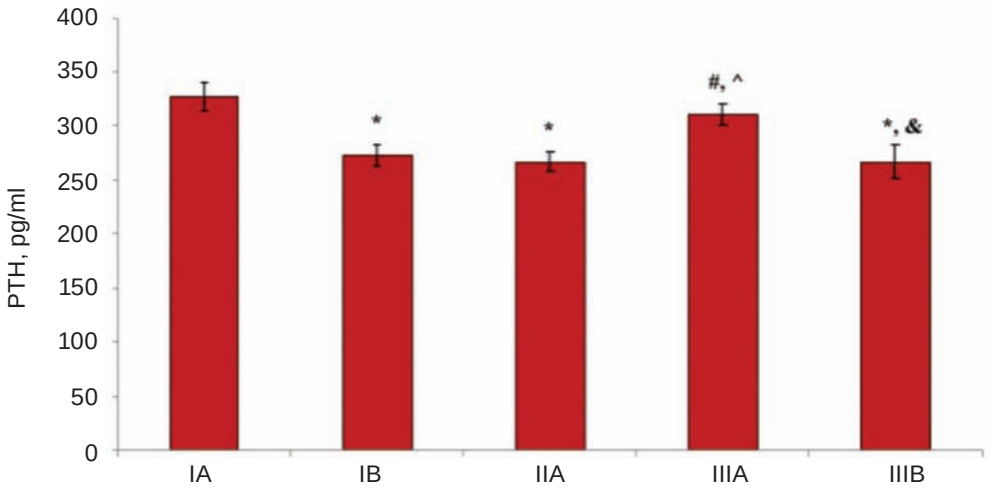

*Fig. 4. Serum PTH levels in the studied groups (M ± m): IA – control group without preeclampsia, IB – control group + preeclampsia, IIA – vitamin D_3-deficiency + without preeclampsia, IIIA – vitamin D_3-deficiency + vitamin D_3+ without preeclampsia, IIIB – vitamin D_3-deficiency + vitamin D_3 + preeclampsia. *Statistically significant difference vs. I A; #statistically significant difference vs. IIA; &statistically significant difference vs. IIIA; ^statistically significant difference vs. IB; $P < 0.05$*

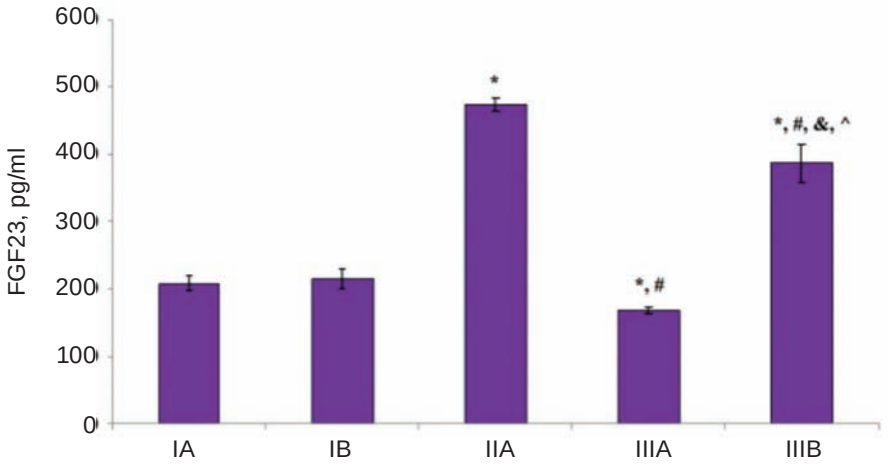

*Fig. 5. Serum FGF23 levels in the studied groups (M ± m): IA – control group without preeclampsia, IB – control group + preeclampsia, IIA – vitamin D_3-deficiency, without preeclampsia, IIIA – vitamin D_3-deficiency + vitamin D_3, without preeclampsia, IIIB – vitamin D_3-deficiency + vitamin D_3 + preeclampsia.*Statistically significant difference vs. IA; #statistically significant difference vs. IIA; &statistically significant difference vs. IIIA; ^statistically significant difference vs. IB; $P < 0.05$*

Vitamin D_3 supplementation led to a significant increase in 25(OH)D_3 levels in Group IIIA, demonstrating the efficacy of the intervention and correlating with its important role in maintaining normal calcium/phosphate metabolism and placental function [27]. At the same time, the decrease in 25(OH)D_3 levels in Group IIIB (supplementation + PE) reflects a partial loss of corrective efficiency under more severe pathological conditions that is in agreement with experimental evidence of disrupted vitamin D_3 metabolism during preeclampsia, particularly due to inflammatory mechanisms and renal dysfunction [28-31].

Hemodynamic disturbances (elevated blood pressure) observed in the groups with vitamin D_3 deficiency and preeclampsia are consistent with studies indicating the role of vitamin D in regulating the renin-angiotensin-aldosterone system, endothelial function, and oxidative stress [30]. Vitamin D supplementation reduced blood pressure (Group IIIA), confirming its vasodilatory and anti-inflammatory effects [32]. However, the excessive increase in blood pressure observed during PE in Group IIIB emphasizes the complex pathophysiological and cellular–molecular mechanisms underlying preeclampsia pathogenesis, when the positive pharmacotherapeutic effects of vitamin D_3 may be limited by the severity of the pathological process [33].

Calcium metabolism exhibited classic features of hypocalcemia under D_3 deficiency and disrupted mineral metabolism during PE, corroborating findings from numerous clinical and experimental studies [34, 35]. The increase in inorganic phosphate levels during PE indicates impaired renal excretion and activation of phosphaturic mechanisms, particularly mediated by hormonal influences of PTH and FGF23.

The classical (calcemic) role of vitamin D_3 is known to be primarily associated with the regulation of calcium–phosphate homeostasis along with two other key hormones produced by endocrine glands – parathyroid hormone (PTH) and calcitonin. Hypocalcemia and hypophosphatemia due to vitamin D_3 deficiency stimulate PTH secretion from the parathyroid glands, leading to secondary hyperparathyroidism. Increased synthesis and release of PTH enhance intestinal calcium absorption, bone mobilization through osteoclast-dependent resorption, and renal tubular reabsorption of calcium, while simultaneously increasing renal phosphate excretion [36-38]. Elevated PTH secretion in response to reduced serum calcium and phosphate additionally induces the expression of mitochondrial cytochrome P450 1α-hydroxylase 25-hydroxyvitamin D (CYP27B1) in proximal renal tubular cells, initiating the synthesis of the biologically active metabolite calcitriol (1α,25$(OH)_2D_3$) – a high-affinity ligand that mediates both endocrine and auto/paracrine actions of this steroid hormone and serves as a potent regulator of phosphate-calcium homeostasis via the

VDR (vitamin D_3 receptor) transcription factor. The presence of secondary hyperparathyroidism may further indicate the development and severity of D_3 deficiency, as well as systemic disturbances in mineral metabolism.

It should be noted that, according to the literature, an increase in PTH levels during early pregnancy is generally a normal physiological adaptation to the additional calcium requirements of the fetus. Early pregnancy may mask symptoms of hyperparathyroidism caused by vitamin D_3 deficiency and hypocalcemia, which may explain the elevated PTH levels observed across all experimental groups in our study, with minor but statistically significant intergroup variations, either above or below those of the intact control group.

The role of FGF23 in the regulation of vitamin D_3 metabolism and mineral homeostasis during normal and pathological pregnancy remains an underexplored area. The alterations in FGF23 observed in our study closely correlate with the pathogenesis of phosphate metabolism disturbances. The significant increase in circulating FGF23 levels during vitamin D_3 deficiency may represent a protective compensatory response aimed at enhancing phosphaturia and reducing tissue calcification under conditions of intensified bone resorption [39].

The elevated FGF23 observed in preeclampsia (Group IIIB) reflects the severity of renal injury and exacerbated systemic inflammation, aligning with studies that identify FGF23 as a biomarker of renal dysfunction and cardiovascular risk [40-42]. Proteinuria, serving as an indicator of glomerular damage, was most pronounced in the PE groups, particularly when concomitant with vitamin D_3 deficiency. This finding is consistent with published literature and our previous data highlighting the role of vitamin D in maintaining renal function and preventing the progression of nephropathy during preeclampsia [43]. These observations underscore the interplay between vitamin D_3 status, phosphate-calcium homeostasis, and renal integrity under conditions of pathological pregnancy.

In summary, vitamin D_3 deficiency exacerbates the course of preeclampsia by intensifying hemodynamic disturbances, elevating FGF23 synthesis, promoting phosphate–calcium imbalance, and impairing renal function. Correction of vitamin D_3 deficiency exerts beneficial effects on the investigated biochemical parameters and vascular function; however, its efficacy is limited in the presence of preeclampsia. These findings are of considerable relevance for future insight into the therapeutic potential of vitamin D_3 for the prevention and comprehensive management of preeclampsia.

Conclusions. This study demonstrated that vitamin D_3 deficiency in pregnant female rats leads to significant disturbances in calcium-phosphate homeostasis, increased serum levels of PTH and FGF23, and the associated activation of mechanisms contributing to hypertension. Vitamin D_3 supplementation partially restored vitamin D_3 status and mineral metabolism, reduced blood pressure, and mitigated renal injury in the absence of L-NAME–induced preeclampsia. However, the development of preeclampsia markedly worsened these parameters, exacerbating arterial hypertension and glomerular damage, highlighting the critical systemic role of vitamin D_3 deficiency in the pathogenesis of preeclampsia. The observed imbalance of regulatory factors governing calcium–phosphate metabolism under conditions of vitamin D_3 deficiency and experimental preeclampsia underscores the complex, multi-level mechanisms of hormonal and metabolic disturbances during pathological pregnancy.

Practical implications. The obtained results suggest the importance of early diagnosis and correction of vitamin D_3 deficiency to prevent the development of severe obstetric complications. Further studies should focus on elucidating the mechanisms of interaction between vitamin D_3 and multiple modulators of vascular–endothelial and renal function under conditions of preeclampsia complicated by vitamin D_3 deficiency and renal pathology.

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ФАКТОР РОСТУ ФІБРОБЛАСТІВ 23 ТА РІВНІ КАЛЬЦІЮ І ФОСФАТУ В СИРОВАТЦІ КРОВІ ЗА УМОВ ЕКСПЕРИМЕНТАЛЬНОЇ ПРЕЕКЛАМПСІЇ: ВПЛИВ СТАТУСУ ВІТАМІНУ D_3

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Прееклампсія (ПЕ) є однією з провідних причин материнської та перинатальної захворюваності, патогенез якої, зокрема, пов'язаний із порушенням мінерального гомеостазу. Останні дані свідчать, що фактор росту фібробластів 23 (FGF23) – ключовий регулятор фосфатного обміну та метаболізму вітаміну D_3 – може відігравати роль у розвитку акушерських ускладнень, однак його значення при ПЕ залишається недостатньо вивченим. Метою даного дослідження було оцінити рівень FGF23 у сироватці крові та його взаємозв'язок із вітаміном D_3 і кальцій-фосфатним балансом при розвитку прееклампсії в експериментальній тваринній моделі. Тридцять п'ять самок щурів лінії Wistar було розподілено на три групи: контрольну (стандартний раціон); щурів із дефіцитом вітаміну D_3; щурів із дефіцитом вітаміну D_3, яким додатково вводили холекальциферол. У кожній групі прееклампсію індукували шляхом введення Nω-нітро-L-аргінін метилового ефіру. Концентрації $25(OH)D_3$, FGF23, паратгормону (ПТГ), загального кальцію, неорганічного фосфату та активність лужної фосфатази (ЛФ) у сироватці крові визначали методом імуноферментного аналізу та біохімічних досліджень. Встановлено, що дефіцит вітаміну D_3 супроводжувався гіпокальціємією, гіпофосфатемією, підвищенням рівня FGF23 та зростанням активності ЛФ у сироватці крові. Додавання вітаміну D_3 призводило до підвищення рівня $25(OH)D_3$, суттєвого зниження концентрації FGF23 та нормалізації мінеральних показників. Індукція прееклампсії зумовлювала виражені порушення кальцій-фосфатного обміну, розвиток артеріальної гіпертензії та 100% летальність тварин із дефіцитом вітаміну D_3. За умов прееклампсії ефективність вітаміну D_3 була обмеженою. У групі ПЕ, незважаючи на застосування вітаміну D_3, рівень FGF23 у сироватці крові залишався значно підвищеним, що свідчить про порушення метаболізму вітаміну D_3. Отримані результати демонструють, що дефіцит вітаміну D_3 посилює тяжкість прееклампсії шляхом порушення мінерального гомеостазу та FGF23-залежної сигнальної регуляції.

Ключові слова: $25(OH)D_3$, дефіцит вітаміну D_3, L-NAME, прееклампсія, кальцій-фосфатний обмін.

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