UNIVERSITY OF MEDICINE AND PHARMACY
"CAROL DAVILA", BUCHAREST
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Correlation of hypovitaminosis D with current maternal-fetal gestational pathology

PHD THESIS SUMMARY

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INTRODUCTION

Recently, vitamin D has received increasing attention and has been associated with multiple health benefits. One area of interest is the effects of vitamin D during pregnancy.

The actions of vitamin D in the body are mediated by the vitamin D receptor that binds to the active form of vitamin D, 1,25 dihydroxy-vitamin D, to induce both transcriptional and non-genomic responses. Vitamin D is well-known for its role in calcium absorption and bone metabolism. Recent studies highlight its importance in modulating the innate and adaptive immune systems and regulating cell proliferation.

Adequate vitamin D intake is essential in pregnancy for the health of the mother and newborn; however, epidemiological data indicate that many pregnant women have healthy serum levels of vitamin D. Hypovitaminosis D during pregnancy has been correlated with the presence of preeclampsia, gestational diabetes, bacterial vaginosis, and an increased risk of cesarean delivery. Several international studies are currently trying to establish how low levels of 25-hydroxyvitamin D (the recognized indicator for assessing vitamin D status) influence the appearance of obstetric complications. Still, a consensus has yet to be reached on this subject.

The literature also emphasizes the link between vitamin D and the placenta during pregnancy. On the one hand, the placenta produces and responds to vitamin D's action, while vitamin D functions as a modulator of its implantation, contributing to the production of cytokines and the adequate immune response in case of infections.

The main objective of the study within the doctoral thesis is to explain the roles of hypovitaminosis D in maternal-fetal gestational pathology and its impact on the health status of the newborn.

The doctoral thesis is structured in two parts: the general part and the personal contribution part. The general part presents the current knowledge regarding vitamin D, its roles in the body, and its specific effects during pregnancy. The personal contribution part includes a doctoral study aimed at correlating hypovitaminosis D with maternal-fetal gestational pathology and its implications on the health status of the newborn.

The doctoral thesis tries to respond to the new links promoted by recent studies by verifying the hypotheses according to which low levels of 25-hydroxyvitamin D are associated with an increased incidence of maternal-fetal pathology, and based on this, the establishment of new research directions.

I. GENERAL PART

1. VITAMIN D – GENERAL CONSIDERATIONS

1.1 Metabolism and structure

Calciferol, or vitamin D, belongs to the class of secosteroid vitamins. They are synthesized from provitamins by cleavage of the B ring from the sterol molecule during exposure of skin tissue to ultraviolet B (UVB) radiation. The most important vitamins D are vitamin D2 (ergocalciferol) and vitamin D3 (cholecalciferol). Both vitamin D2 and vitamin D3 do not have significant biological activity, so their metabolism into hormonally active forms is necessary. The activation of vitamin D takes place under the action of specific enzymes in two stages, first at the liver level and then at the renal level. The main circulating reservoir and the best indicator of the body's overall status of vitamin D is 25-hydroxyvitamin D.

1.2 Roles of vitamin D in the body

Vitamin D plays an essential role in bone development, maintaining bone integrity, and the functioning of the neuromuscular system. In addition to these well-known roles, it has multiple beneficial effects on the human body, with vitamin D's actions including adaptive and innate immune systems, pancreatic β cells, heart and cardiovascular system, and the brain system [1]. The impact on these tissues includes effects on hormone secretion, modulation of immune responses, and control of cell proliferation and differentiation. Thus, vitamin D analogs can prove helpful for preventing and treating certain conditions.

1.3 Normal values and vitamin D requirements

More and more international organizations have proposed establishing the threshold for optimal serum levels of 25-hydroxyvitamin D of 30 ng/ml. According to the Society of Endocrinology, vitamin D status is defined as mild insufficiency/deficiency 20-29 ng/ml, moderate deficiency 10 – 19 ng/ml and severe deficiency <10 ng/ml.

1.4 Hypovitaminosis D in pregnancy

Hypovitaminosis D represents serum 25-hydroxyvitamin D levels less than 30 ng/ml, subclassified as vitamin D insufficiency or deficiency.

Vitamin D deficiency in pregnant women is associated with an increased risk of complications during pregnancy [108]. These include preeclampsia, intrauterine fetal growth restriction, small fetuses for gestational age, bacterial vaginitis, and gestational diabetes mellitus [109][110]. Maternal vitamin D deficiency has also been linked to adverse effects in newborns, including reduced bone density, rickets throughout childhood, as well as an increased risk of developing asthma and schizophrenia [111-114].

Studies have shown that a balanced diet is insufficient to cover the pregnant woman's need for vitamin D, iron, and folic acid. Thus, although the daily requirement for vitamin D amounts to at least 5 micrograms, dietary intake usually does not exceed 2–2.2 micrograms per day [5][6]. This is also confirmed by the fact that, quite commonly, a low level of 25-OH vitamin D is detected in pregnant women – the most widely accepted indicator for vitamin D status, regardless of its source. The problem of vitamin D deficiency during pregnancy is a reality, including in developed countries, where nutritional rickets have a low incidence.

Pregnant women are recommended to have an optimal circulating 25-OHD level of at least 40 ng/ml from the beginning of pregnancy. [7] Studies have shown that it must provide maximum protection against pregnancy complications, including preeclampsia, or against certain conditions, such as the onset of asthma in the newborn. It is important to consider that 25-hydroxyvitamin D levels can be influenced by the season, the degree of exposure to sunlight, and food intake.

1.5 Vitamin D receptor in pregnancy

The presence of VDR at the placental level suggests that vitamin D has a direct effect on specific tissues at the maternal-fetal interface [15]. One possible explanation is that 1,25(OH)2D acts as a regulator of placental calcium transport, but the immunomodulatory role in the placenta has also been proposed [15][16]. In addition, the rapid expression of VDR and CYP27B1 early in pregnancy suggests that vitamin D may play a fundamental role in the placenta's conception, implantation, and development [17]. Also, vitamin D has an essential role in vascular development, neovascularization with inhibition of placental neoangiogenesis and in regulating perivascular support [18].

1.6 Vitamin D and placenta

1.6.1 Placentation process

Vitamin D regulates critical target genes associated with implantation at the trophoblast level, promotes immunosuppression, and induces decidualization. It also regulates the myometrial contractile profile and proliferation of myometrial cells, promotes EVT invasion, and antibacterial and anti-inflammatory responses. Vitamin D also increases anti-inflammatory and anti-migration activity and regulates the production of hCG, hPL, estradiol, and progesterone. These processes demonstrate vitamin D's direct role in antibacterial/anti-inflammatory responses.

1.6.2 Placental function

The roles played by vitamin D in implantation and placentation are well established, with placental vitamin D receptor (VDR) expression considered a critical regulator of placental and fetal growth [120]. Vitamin D is also thought to regulate genes involved in placental development [121] significantly. In addition, 1,25(OH)2D is proven to increase the availability of vascular endothelial growth factor (VEGF)[122]. According to the literature, pregnant women with 25(OH)D levels below 20 ng/ml have low serum placental growth factor (PIGF) values, which in turn could lead to preeclampsia and intrauterine fetal growth restriction [123]

2. VITAMIN D AND MATERNAL-FETAL GESTATIONAL PATHOLOGY

2.1 Miscarriage

It is estimated that 31% of pregnancies end in miscarriage, with two-thirds of losses not being clinically diagnosed [20]. Studies have shown that women who had a normal pregnancy and birth had significantly higher levels of vitamin D than those who experienced miscarriages [21] and that adequate vitamin D levels protect against miscarriages [22]. Compared to women with recurrent miscarriages and normal vitamin D levels, women with recurrent miscarriages and hypovitaminosis D have an increased prevalence of autoimmune and cellular abnormalities [22].

2.2 Genital infections – bacterial vaginosis

Vitamin D deficiency is associated with an increased risk of bacterial vaginosis [80]. Although this cannot interfere with the ability to conceive, it causes discomfort to the woman and

poses a threat to the viability of a pregnancy [81]. Bacterial vaginosis is also associated with reproductive failure, miscarriage, premature rupture of membranes and premature birth, chorioamnionitis, and postpartum endometritis [81].

2.3 Gestational diabetes

Several studies claim that hypovitaminosis D can negatively affect pregnant women, among other things, by inducing gestational diabetes, and therefore can affect fetal growth, favoring macrosomia [46][48]. The mechanisms underlying this hypothesis are the effects of vitamin D on insulin secretion in pancreatic beta cells.

2.4 Preeclampsia

Vitamin D deficiency not only increases the risks of preeclampsia but also negatively affects the development of the child in extrauterine life [60]. The incidence of preeclampsia correlates inversely proportionally with serum 25(OH)D levels. One study found a five-fold increase in cases of preeclampsia in pregnant women with vitamin D levels below 15 ng/ml compared to pregnant women with normal vitamin D levels [61]. Another study reported a 27% reduction in the risk of preeclampsia in women who took vitamin D supplements as opposed to those who did not receive vitamin D supplementation [55].

2.5 Intrauterine growth restriction

Vitamin D concentration in the placenta and placental vitamin D receptor (VDR) expression have recently been shown to be low in IUGR and contribute to trophoblastic dysfunction [156]. Therefore, decreased placental VDR expression may affect or limit the beneficial actions and effects of maternal/placental vitamin D in regulating fetal placental growth.

2.6 Preterm birth

Vitamin D could influence the physiology of preterm birth through its action on inflammation and immunomodulatory processes [67]. The effect of vitamin D on the proper function of toll-like receptors that initiate the innate immune response has been demonstrated. Several studies have tried to identify the correlation between vitamin D status and the incidence of preterm birth, but the results are contradictory.

2.7 Cesarean section

It is considered that maternal vitamin D deficiency also leads to an increased risk of completing the birth by cesarean section. One study reported an approximately four-fold increase in the incidence of cesarean section in women with a 25(OH)D level below 15.2 ng/ml compared to women with a 25(OH)D above 15.2 ng/ml. One possible reason for the potentially higher risk of completing birth by cesarean section in women with lower vitamin D concentrations was reduced pelvic muscle power, leading to prolonged labor [75].

II. PERSONAL CONTRIBUTIONS

CORRELATION OF HYPOVITAMINOSIS D WITH CURRENT MATERNO-FETAL GESTATIONAL PATHOLOGY

3. WORKING HYPOTHESIS AND OBJECTIVES

We chose this research topic based on the hypothesis that hypovitaminosis D affects the processes involved in pregnancy, thus increasing the risk for maternal and fetal complications.

This thesis aims to prove the link between maternal vitamin D status and the presence of gestational pathology and to determine, depending on maternal values, whether vitamin D deficiency is present in the newborn**.**

Specific objectives

1. Establishing the incidence of hypovitaminosis D in pregnancy
2. Correlating hypovitaminosis D with demographics
3. Correlation of hypovitaminosis D with maternal-fetal gestational pathology
4. Correlation of maternal hypovitaminosis D with fetal serum vitamin D levels
5. Correlation of maternal hypovitaminosis D with fetal parameters at birth
6. Correlation of the results obtained with the literature
7. Establishing new research directions based on the results obtained

This research within the doctoral thesis aims to represent a first step in determining the effects of vitamin D deficiency in pregnancy on the mother and newborn and identifying strategies to prevent maternal-fetal gestational complications.

Hypovitaminosis D is currently a public health problem, with an increased incidence both nationally and internationally. More and more studies are trying to explain how low serum vitamin D levels affect various systems in the body, but without reaching a consensus.

4. GENERAL RESEARCH METHODOLOGY

Several statistical methods to analyze and compare the results were used in my doctoral thesis.

For the statistical analysis, I began with a descriptive part, where I calculated the parameters of central tendency and dispersion for the numerical variables, ran the frequency tables for the ordinal and nominal variables, and extracted the main percentages. Following the results obtained, I established correlations between the analyzed variables.

The data were graphically represented using histograms, line/column graphs, boxplots, and pie charts.

The Shapiro-Wilk test was applied to examine the distribution of data. For a unitary organization of the results, non-parametric tests were used, such as Mann-Whitney, for two different samples and Kruskal-Wallis for more than two distinct groups. The associations between the studied variables were tested using the Chi-square test.

In addition to the statistical tests, we performed the risk analysis, calculating the relative risk parameters (RR) and odds ratio (OR) for the given sample and estimated the 95% confidence interval to determine whether the mother's vitamin D deficiency has implications for vitamin D values in the newborn.

At the end of the study, a linear regression analysis was applied to demonstrate the link between vitamin D levels in mothers and newborns and gestational pathology. For the entire thesis, the confidence level was set at $\alpha=0.05$.

For the statistical analysis, I used the SPSS Statistics 29 program and graphically represented the results using Microsoft Office Excel 2019.

5. MATERIAL AND METHOD

To carry out the doctoral thesis, I conducted a retrospective, observational, analytical study within the Clinical Hospital of Obstetrics and Gynecology Prof. Dr. Panait Sârbu after obtaining the opinion of the Hospital's Ethics Commission, in accordance with the Helsinki Declaration of 1975 and with the national legislation. The research for the doctoral study was carried out over a period of 3 years, between November 2020 and November 2023.

After applying the inclusion and exclusion criteria, the study analyzed 130 pregnant patients and 130 newborns.

Inclusion criteria were as follows: age range 16-45 years, average of origin (patients from the same geographical region were included to avoid regional variations in vitamin D status), single pregnancy, and complete medical records (demographic information, complete medical history, vitamin D status, biological profile)

The exclusion criteria were the following: pre-existing medical conditions—chronic kidney disease, malabsorption syndrome, use of chronic treatments that could affect vitamin D metabolism—anticonvulsants, corticosteroids, multiple pregnancies, and incomplete medical records.

The numerical variables that are the basis of the statistical research were established based on the objectives pursued. The numerical variables used in the study were maternal age (years), maternal serum 25-hydroxyvitamin D values, weight, height, body mass index, systolic blood pressure, diastolic blood pressure, hemoglobin values, blood glucose values, gestational age at birth (weeks), newborn weight, Apgar score and serum 25-hydroxyvitamin D values newborn.

The observation medical records were analyzed to identify and extract all the information necessary for statistical analysis.

6. RESULTS

The database, which contains 260 patients, 130 pregnant women, and 130 newborns, after applying the inclusion and exclusion criteria, was initially divided into two:

- Pregnant patients without vitamin D deficiency (35 patients – 26.92%) – control group
- Pregnant patients with vitamin D deficiency (95 patients – 73.08%) – study group.

Based on the degrees of vitamin D deficiency, the patients were grouped as follows, according to international and national recommendations regarding serum vitamin D levels: severe deficiency (below 10 ng/ml) – 12 patients (9.23%); moderate deficiency (between 10 - 20 ng/ml) – 47 patients (36.15%); mild deficiency (between 21 - 29 ng/ml) – 36 patients (27.7%); and 35 patients (26.92%) with optimal vitamin D levels (between 30 - 100 ng/ml).

Vitamin D levels were also measured in newborns, obtaining: severe deficiency (below 10 ng/ml) – 5 subjects (3.85%); moderate deficiency (between 10 - 15 ng/ml) – 19 subjects (14.62%); mild deficiency (between 16 - 19 ng/ml) – 50 subjects (38.46%); and 56 patients (43.07%) with optimal vitamin D levels (between 20 - 100 ng/ml).

Patients will be divided according to these two classifications for the entire study.

6.1 Incidence of maternal hypovitaminosis D in the study group

Following the analysis of vitamin D status in the study group composed of pregnant patients, we can observe an increased incidence of hypovitaminosis D, 64%, compared to patients with normal vitamin D values, 36%.

6.2 Study lot distribution by vitamin D status and demographic data

Following the study group analysis according to the environment of origin, we identified 34 patients (26.15%) from rural areas and 96 patients (73.85%) from urban areas.

We can note an increased incidence of hypovitaminosis D (mild, moderate, or severe deficiency) in both rural and urban areas. In rural areas, only 26.47% of patients have oppressed serum levels of vitamin D, and in urban areas, 27.08% have an adequate vitamin D status.

6.3 Correlation of hypovitaminosis D with gestational pathology

Depending on the presence of gestational pathologies and maternal vitamin D status, we performed a batch analysis for each condition.

6.3.1 Correlation of hypovitaminosis D with maternal anemia

A direct link between them can be observed based on the analysis of the incidence of anemia according to vitamin D status. Anemia was diagnosed in 58.3% of cases associated with mild vitamin D deficiency, 53.19% of the cases related with moderate vitamin D deficiency and 66.6% of cases associated with severe deficiency.

We can also note the absence of anemia in cases with optimal vitamin D values, with a 91.4% percentage.

6.3.2 Correlation of hypovitaminosis D with gestational diabetes

Following the analysis of the incidence of gestational diabetes according to vitamin D status, we identified the following: patients with mild deficiency were diagnosed with GD in 11.1% of cases, those with moderate deficiency presented with GD in 21.2% of cases, and those with severe deficiency presented with GD in 25% of cases.

Patients with optimal vitamin D values were diagnosed with GD in only 8.5% of cases.

6.3.3 Correlation of hypovitaminosis D with gestational hypertension

Following the analysis of the incidence of gestational hypertension according to vitamin D status, we observed the following: patients with mild deficiency were diagnosed with gestational hypertension in 13.8% of cases, those with moderate deficiency had gestational hypertension in 17% of cases, and those with severe deficiency were diagnosed with gestational hypertension in 25% of cases. It is important to note that patients with optimal vitamin D values have not been diagnosed with gestational hypertension.

6.3.4 Correlation of hypovitaminosis D with preeclampsia

Following the analysis of the incidence of preeclampsia according to vitamin D status, it was observed that patients were diagnosed with preeclampsia in only 11.1% of cases with mild vitamin D deficiency.

6.3.5 Correlation of hypovitaminosis D with thrombophilia

Following the analysis of the incidence of thrombophilia according to vitamin D status, it was identified that thrombophilia was present in 8.3% of cases with mild vitamin D deficiency, in 4.2% of cases with moderate vitamin D deficiency, and in 8.3% of cases with severe deficiency. In patients with optimal vitamin D values, thrombophilia was present in 11.4% of cases.

6.4.6 Correlation of hypovitaminosis D with autoimmune thyroiditis

Following the analysis of the incidence of autoimmune thyroiditis according to vitamin D status, it was identified that autoimmune thyroiditis was diagnosed in 13.8% of cases with mild

vitamin D deficiency and 6.3% of cases with moderate deficiency. No cases of autoimmune thyroiditis have been identified among patients with severe deficiency. Patients with optimal vitamin D values experienced autoimmune thyroiditis in 22.8% of cases.

6.3.7 Correlation of hypovitaminosis D with genital group B streptococcus infection

Following the analysis of the incidence of GBS infection according to vitamin D status, the following was identified: among patients with mild and moderate vitamin D deficiency, GBS infection was diagnosed in 8.3% and 10.6% of cases, respectively. There were no cases of GBS infection among patients with severe deficiency or optimal vitamin D levels.

6.3.8 Correlation of hypovitaminosis D with urinary tract infection

Following the analysis of the incidence of urinary tract infection according to vitamin D status, we identified the following: patients with mild vitamin D deficiency had urinary tract infection in 11.1% of cases, those with moderate deficiency were diagnosed with urinary tract infection in 8.5% of cases, and those with severe deficiency had urinary tract infection in 16.6% of cases. Patients with optimal vitamin D values were diagnosed with urinary tract infections in 8.5% of cases.

6.4 Incidence of hypovitaminosis D in neonates in the study group

Following the analysis of the vitamin D status in the study group composed of newborns, we can observe an increased incidence of hypovitaminosis D, 57%, compared to those with normal vitamin D values, 43%.

The vitamin D status in newborns was represented as follows: severe deficiency (below 10 ng/ml) – 5 subjects (3.85%); moderate deficiency (between 10 - 15 ng/ml) – 19 subjects (14.62%); mild deficiency (between 16 - 19 ng/ml) – 50 subjects (38.46%); and 56 patients (43.07%) with optimal vitamin D levels (between 20 - 100 ng/ml).

6.5 Correlation of maternal vitamin D status with the sex of newborns

Regarding the gender distribution of newborns correlated with vitamin D status, it can be concluded that sex is not a factor that influences hypovitaminosis D, which has an almost equal distribution according to sex.

6.6 Correlation of hypovitaminosis D in newborns with maternal vitamin D status

In cases of mild vitamin D deficiency in the mother, newborns showed mild vitamin D deficiency in 42% of cases and optimal vitamin D values in 58% of cases. In cases of moderate vitamin D deficiency in the mother, newborns experienced mild deficiency in 74% of cases and moderate deficiency in 26% of cases. In cases with severe vitamin D deficiency in the mother, newborns had moderate deficiency in 58% of cases and severe deficiency in 42%. In cases with optimal vitamin D status in the mother, all newborns had optimal vitamin D values. Following the results obtained, we can observe the direct correlation between the vitamin D status in the mother and the serum vitamin D values in the newborn.

6.7 Statistical analysis of the study lot

For the numerical variables: maternal age, vitamin D values in mothers and newborns, maternal body mass index (BMI), gestational age (VG), newborn weight (NN), APGAR score, systolic blood pressure (SBP), diastolic blood pressure (DBP), hemoglobin (Hb), and blood glucose, we calculated the central tendency and dispersion parameters, first for the entire sample and then dividing the data according to the degrees of vitamin D deficiency.

Table 6.16: Descriptive statistics of the numerical variables included in the study

Statistical analysis	Mother's age	Vitamin D values	BMI	GP	New born weight	Apgar Score	New born Vitamin D values	SBP	DBP	Hemoglobin	Blood sugar
Valid data	**130**	**130**	**130**	**130**	**130**	**130**	**130**	**130**	**130**	**130**	**130**
Mode	36	34.25	20.9	38	2980	9	21.34	117	70	11.6	72
Median	33	20.69	29.22	38	3135	9	16.95	121	78	11.6	76.61
Mean	32.42	22.43	29.16	38	3107.3	9	20.33	121.7	78.5	11.6	81.58
Standard deviation	6.02	9.38	5.55	1.94	544.2	0.9	7.98	11.4	9.57	1.45	20.88
Standard error of mean	0.53	0.82	0.49	0.17	47.73	0.08	0.7	1	0.84	0.13	1.83
p value from Shapiro - Wilk	0.015	<0.001	0.08	<0.001	0.06	<0.001	<0.001	0.01	0.04	<0.001	<0.001
Range	29	34.49	24.53	11.2	296	5	44.32	67	49	8.3	191
Minimum	16	5.2	18.07	29	143	5	5.07	88	56	6.2	55
Maximum	45	39.71	42.6	40.2	439	10	49.39	155	105	14.5	246

6.8 Statistical analysis of the study plot according to vitamin D status

Depending on the maternal vitamin D status, we performed a detailed statistical analysis of the variables presented above.

The results were presented in detail and represented by boxplot graphs and histograms.

6.9 Statistical analysis - Mann test – Whitney U

Since the groups studied have non-normal distributions, we applied statistical tests to identify the significance of the data obtained. First, the Mann–Whitney U test was used to see if there were significant differences between the numerical variables studied in the presence or absence of vitamin D deficiency.

Significant results ($p<0.05$) were obtained in BMI, APGAR score, in the mean values of vitamin D of the newborn, and in the mean values of hemoglobin and blood glucose of the mother, in all cases patients with normal values of vitamin D had better medical outcomes.

Table 6.18 Mann-Whitney U Test

Variables	**W**	**p**
Maternal age	1997.500	0.078
BMI	1212.000	**0.018**
Gestational age	1939.000	0.138
Newborn weight	1587.000	0.694
APGAR score	1164.500	**0.003**
Newborn serum vitamin D values	3041.500	**< .001**
SBP	1392.500	0.157
SBD	1434.500	0.232
Hemoglobin	2651.000	**< .001**
Blood sugar	932.000	**< .001**

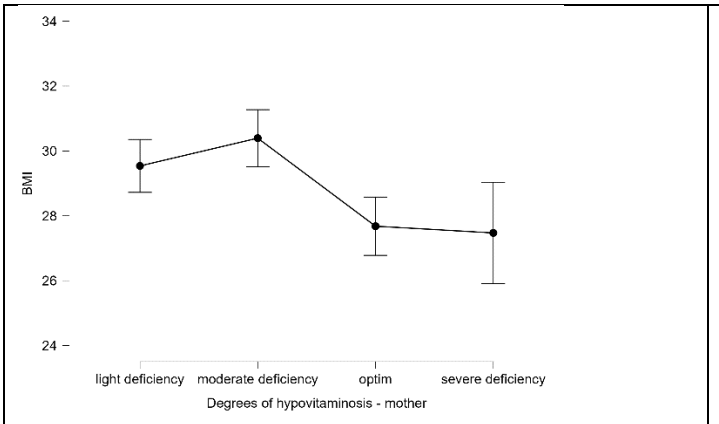
Following the statistical analysis, the following correlations were identified:

- maternal vitamin D status and body mass index, $p = 0.018$
- maternal vitamin D status and APGAR score, $p = 0.003$
- maternal vitamin D status and serum vitamin D values in the newborn, $p < .001$

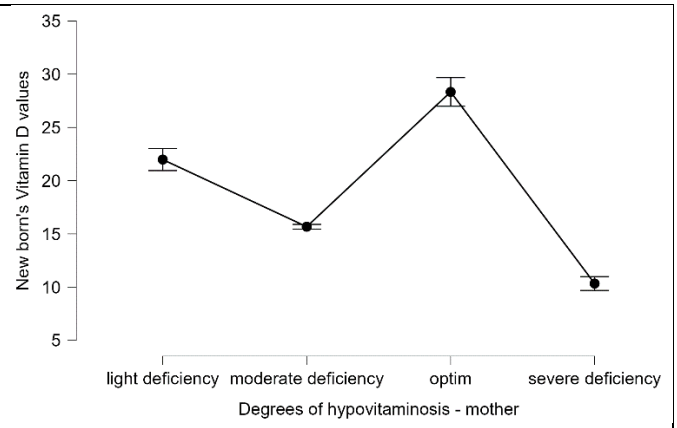
- maternal vitamin D status and serum maternal hemoglobin values, p <.001
- maternal vitamin D status and serum blood glucose values, p < .001

6.10 Statistical analysis - Kruskal – Wallis test

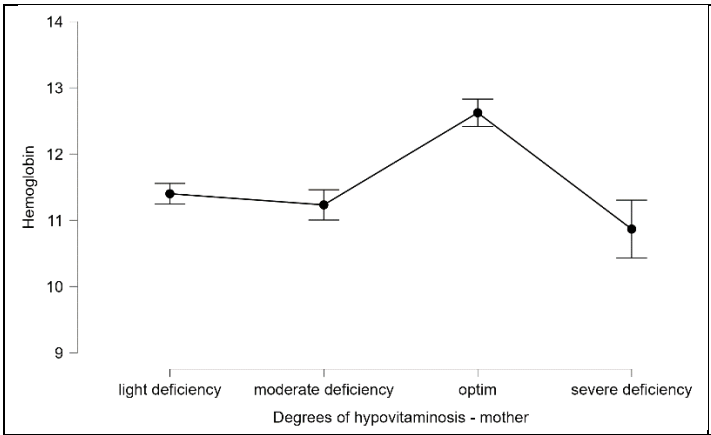
Based on the obtained results, we tested the significant results based on the degrees of vitamin D deficiency using the Kruskal – Wallis test. So, for BMI, we got significant results (p=0.035, Statistic=7.7); the lowest BMI was seen in the patients with severe vitamin D deficiency. One of the most important results obtained up to this point was that the children of the patients with severe vitamin D deficiency also have severe vitamin D deficiency in most cases (p<0.001, Statistic=96.686). As well, as in the previous study, in the case of Kruskal – Walli's test, we obtained significant results in the case of hemoglobin (p<0.001, Statistics=27.821) and blood sugar (p=0.002, Statistics=15.126). As can be seen from the following figure, women with a severe deficit of vitamin D have severe anemia, as well as high blood sugar values.



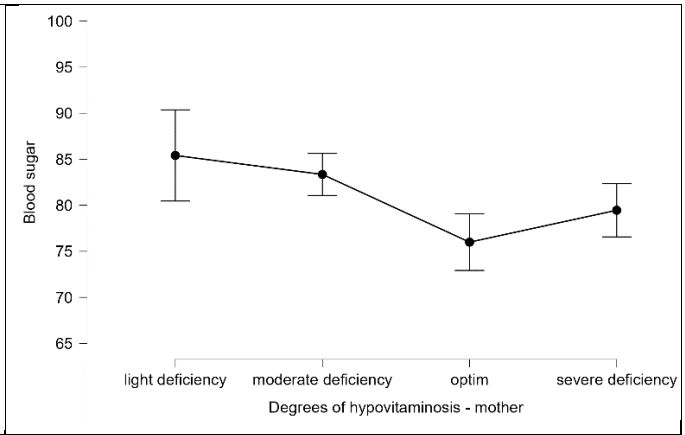
a. Maternal BMI according to maternal vitamin D status



b. Newborn vitamin D serum values according to maternal vitamin D status



c. Maternal serum Hb values according to maternal vitamin D status



d. Maternal serum blood glucose values according to maternal vitamin D status

Fig. 6.44 Graphical representation of the Kruskal – Wallis test

6.11 Determination of risk associated with maternal hypovitaminosis D

The central part of this analysis was testing to see if the mother's deficiency in vitamin D can be considered as a risk factor for the newborn, so for that, the data was split into a contingency table, obtaining extremely significant risk factor ($p < 0.001, RR > 1, OR > 1$), so it can be concluded that if the mother has vitamin D deficiency, it increases more than eight times the chance that the newborn will have a vitamin D deficit. The Chi-square test was used in this case.

Table 6.19 Determination of risk associated with maternal hypovitaminosis D

Contingency Table			Results
Variables	Newborn with vitamin D deficiency	Newborn without vitamin D deficincy	$p < 0.001$ $RR = 8.84$, 95% $CI \in (2.98; 26.25)$ $OR = 33.39$, 95% $CI \in (9.35; 119.28)$
Mother with vitamin D deficiency	**72**	**23**	
Mother without vitamin D deficiency	**3**	**32**	

Given these results, further tests were performed to observe whether hypovitaminosis D can increase the mother's risk of developing various pathologies in pregnancy, and significant results were obtained $p<0.05$ in the following cases:

- pregnancy-induced hypertension, p=0.048
- Autoimmune thyroiditis, p=0.037
- preeclampsia, p=0.013
- anemia, p=0.027

As a result, we can say that hypovitaminosis D can increase the risk of specific pathologies during pregnancy.

At the end of the thesis a linear regression model was run to see if an association between the mother's and newborn vitamin D levels exits, obtaining a significant, strong, positive direct correlation $(r = 0.795,\ R^2 = 0.632, p < 0.001)$. Meaning that these two medical tests are extremely dependent one on the other.

DISCUSSIONS

Adequate vitamin D intake is crucial for maternal and fetal health during pregnancy. However, epidemiological data indicate that many pregnant women have low serum levels of 25-hydroxyvitamin D (25-(OH)D). Some studies have tried to establish a link between low levels of 25-hydroxyvitamin D (vitamin D status barometer) and the occurrence of obstetric complications. Still, so far, there is no consensus on this matter. Pregnant women tend to develop vitamin D deficiency, reflected in maternal and fetal serum levels of 25-hydroxyvitamin D, which should typically range from 30 to 100 ng/ml for adequate status. [5] [6].

Following the study within the doctoral thesis, we also noticed an increased incidence of hypovitaminosis D among pregnant women in the study group. Of these, only 26.92% had an optimal vitamin D status, while 73.08% had hypovitaminosis D in various stages.

Regarding the supplementation of vitamin D intake, it is essential to mention the significant differences between the multivitamin supplements recommended during pregnancy. Following the analysis of the doses of vitamin D present in the three most common types of multi-vitamin supplements administered during pregnancy in Romania, we identified that in all three cases, the doses of vitamin D had values below 1000 IU (200 IU, 250 IU, respectively 800 IU). Thus, no

multivitamin supplement complies with the recommendations of international studies to ensure a minimum IU intake of vitamin D.

This finding may explain the increased incidence of vitamin D deficiency during pregnancy, even in cases where supplementation is taken.

Several clinical trials have documented an association between low 25(OH)D levels and adverse pregnancy outcomes, such as pregnancy-induced hypertension, gestational diabetes, miscarriage, preterm birth, and postpartum depression. These studies also highlight the impact of vitamin D deficiency on placental circulation [13-16].

Regarding the incidence of anemia in pregnant women included in the study group, following the statistical analysis, we highlighted a direct link between vitamin D status and the presence of anemia ($p=0.027$). Also, the severity of anemia was directly proportional to the degree of hypovitaminosis D.

The literature also confirms these results. A 2022 meta-analysis that included eight studies and 6530 pregnant women identified that maternal hypovitaminosis D increases the risk of anemia in pregnancy by 61%

Vitamin D deficiency is associated with an increased risk of gestational diabetes [44][50]. Given the positive effects of 1,25(OH)2D on increased insulin sensitivity and insulin production, the association between hypovitaminosis D and gestational diabetes is unsurprising [51].

As a result of the current study, we also identified a direct correlation between vitamin D status and blood glucose values in pregnant women ($p<0.001$).

Given the well-known link between gestational diabetes and body mass index, we also looked at how vitamin D status influences this index in the study. Thus, we identified that hypovitaminosis D is statistically significantly correlated with BMI ($p=0.018$).

Several studies have found a significant link between low serum vitamin D levels and preeclampsia, showing that for every 10 nmol/ml (4 ng/ml) increase in blood 25(OH)D levels, the likelihood of severe preeclampsia decreased by 38%.

The present study's results also demonstrated a direct correlation between the presence of hypovitaminosis D, the incidence of preeclampsia ($p=0.013$), and the development of gestational hypertension ($p=0.048$).

In terms of the modulatory effects of the immune system, serum vitamin D levels were associated with the onset and progression of several autoimmune diseases, including autoimmune

thyroiditis, a pathology also analyzed in the present study. The study identified a direct association of hypovitaminosis D with the presence of autoimmune thyroiditis (p=0.037).

Another topic of interest is the link between maternal vitamin D status and newborn health. It is recognized that vitamin D is essential for fetal skeletal development and normal growth. Some studies suggest that prenatal vitamin D deficiency is associated with lower birth weight, small-for-gestational-age newborns, and intrauterine growth restriction [18].

Following the study group analysis, we did not identify a statistically significant relationship between serum vitamin D values and newborn birth weight (p = 0.694).

Regarding the condition of the newborn, the results of the study in the doctoral thesis correlated the presence of maternal hypovitaminosis D with the values of the APGAR score (p=0.003), thus highlighting the importance of an optimal maternal vitamin D status for a better adaptation of the child to extrauterine life.

International studies also confirm the results obtained from correlating the incidence of hypovitaminosis D in the newborn with the maternal vitamin D status. A 2017 study conducted in a third-degree maternity hospital in northern India established a direct correlation between the serum values of 25-(OH)D of newborns and maternal serum values, with a statistical significance of p = 0.001. At the same time, we concluded that hypovitaminosis D in the mother increases the risk of vitamin D deficiency in the newborn by eight times.

Given the multiple correlations between hypovitaminosis D and gestational pathology and its impact on the health status of the newborn, further studies on larger groups of patients are needed to have a clearer picture of vitamin D's effects and mechanisms of action during pregnancy.

Regarding the limitations of the current study, it is worth mentioning the relatively small group of pregnant patients, 130, compared to other international studies that analyzed larger cohorts. Other limitations of the study are its retrospective type and the fact that it was conducted in a single clinic.

However, so far, in the Romanian literature, we have not identified any other research aimed at the correlation between maternal hypovitaminosis D and gestational pathology and its impact on newborn parameters. Thus, this study can represent a first step in establishing research directions, including conducting randomized clinical trials on large batches of patients.

8. CONCLUSIONS AND PERSONAL CONTRIBUTIONS

Conclusions

The doctoral research "Correlation of hypovitaminosis D with current maternal-fetal gestational pathology" aimed to identify the effects of vitamin D status during pregnancy and on the health parameters of newborns.

The objectives of the doctoral thesis were fulfilled by conducting a comprehensive study, which analyzed the incidence of hypovitaminosis D among pregnant women, correlations of vitamin D status with pathologies such as anemia, gestational diabetes, gestational hypertension, preeclampsia, autoimmune thyroiditis, and presence of genitourinary infections. The study also looked at the link between maternal vitamin D status and hypovitaminosis D in newborns and the impact on their health.

The study's results identified statistically significant correlations between maternal hypovitaminosis D and the risk of developing anemia, gestational hypertension, preeclampsia, and autoimmune thyroiditis during pregnancy. As a result of the research, we identified maternal vitamin D deficiency is a risk factor for newborns, with maternal hypovitaminosis D increasing the newborn's risk of deficiency by eight times. At the same time, the current study also showed the effects of maternal vitamin D status on the newborn's adaptation process to extrauterine life.

Regarding the current study's limitations, it is worth mentioning the relatively small group of pregnant patients, respectively 130, compared to other international studies that analyzed larger cohorts. Other limitations of the study are its retrospective type and the fact that it was conducted in a single clinic.

The impact of vitamin D on gestational pathology is a significant research topic, and randomized clinical trials on large groups of patients are needed to have a more complete picture of its mode of action and effects. Given the risks associated with gestational pathology, both maternal and fetal, it is essential to determine the risk factors for these conditions to develop preventive strategies.

Despite numerous studies that have looked at the effects of vitamin D in pregnancy, there is currently no consensus on its mechanisms of action. Also, it is not possible to speak clearly of an optimal level of vitamin D in pregnancy, but ensuring an adequate intake through a balance between a healthy diet, safe and limited exposure to the sun as well as the administration of supplements containing D, can contribute to a healthy pregnancy, with long-term beneficial effects,

both for the mother and the fetus. According to the guidelines, it is recommended that pregnant women monitor their serum 25-hydroxyvitamin D levels during pregnancy.

This doctoral thesis can contribute to improving medical practice by signaling the importance of vitamin D in pregnancy and establishing new research directions.

Personal contributions

As for the personal contribution, I can say that:

- this paper represents the first research in Romania that analyzed the impact of vitamin D on both the pathology associated with pregnancy and the parameters of the newborn
- I argued the need for an optimal vitamin D status during pregnancy
- I found an increased incidence of hypovitaminosis D during pregnancy
- I have demonstrated the correlation between maternal vitamin D status and body mass index
- I have demonstrated the correlation between the presence of maternal hypovitaminosis D and serum hemoglobin values, implicitly the appearance of anemia
- I have demonstrated the correlation between the presence of maternal hypovitaminosis D and serum blood glucose values
- I have shown that maternal hypovitaminosis D increases the risk of developing pregnancy-induced hypertension and preeclampsia
- I have demonstrated the correlation between hypovitaminosis D and the presence of autoimmune thyroiditis
- Starting from maternal serum vitamin D levels, I demonstrated the correlation between these and serum 25-hydroxyvitamin D values among newborns
- I have identified that maternal hypovitaminosis D increases the risk of vitamin D deficiency in the newborn by eight times
- I demonstrated the correlation between maternal hypovitaminosis D and APGAR score in the newborn
- I compared the results obtained with data from the literature for a clearer highlighting of the correlation between hypovitaminosis D and gestational pathology
- Based on the results obtained, I have established new research directions on the effects of vitamin D in pregnancy

- I have highlighted, by correlating the representative values for maternal hypovitaminosis D with gestational pathology and hypovitaminosis D in the newborn, the fact that supplementation with vitamin D among pregnant women is required, between 1000-2000 UI daily, dose which is not contained by many vitamin supplements frequently recommended in Romania
- I have objectified the gestational conditions that can be prevented by vitamin D supplementation during pregnancy, representing valuable data for the composition of a high-performance pregnancy surveillance program with maternal-fetal health and economic benefits for the Romanian health system.

The study carried out within the doctoral thesis brings novelty elements to medical practice in Romania through the contributions resulting from the medical statistical analysis of the correlations between hypovitaminosis D and maternal-fetal gestational pathology. It represents a first step in establishing future research directions for preventing complications during pregnancy.

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