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Features of the concentration of vitamin and mineral compounds in women with antenatal fetal death in anamnesis at the pre-pregnancy stage

Tetiana Ilnytska[1], Vasyl Beniuk[1], Nazarii Hychka[1,2], Viktor Oleshko[1], Antonina Chebotarova[1]

[1] Department of Obstetrics and Gynecology No. 3, Bogomolets National Medical University, Kyiv, Ukraine

[2] Maternity and Childhood Center of the Municipal Non-Profit Enterprise «Kyiv City Clinical Hospital No. 5»

Address for correspondence:

Antonina Chebotarova

E- mail: doctortonya@ukr.net

Abstract: *Antenatal fetal death is one of the most severe obstetric condition, which has a significant impact on both somatic health and the psycho-emotional state of the woman. Recently, the data appeared that highlight the role of vitamin-mineral balance in the antenatal fetal death etiopathogenesis due to the influence on the angiogenesis and on the formation of placental blood flow processes. Therefore, studying the vitamin-mineral compounds levels in women with a history of antenatal fetal death is particularly relevant and prompted the formation of our study purpose: to determine the features of the folic acid, vitamins B, D, ferritin, homocysteine concentrations in women with antenatal fetal death in anamnesis at the stage of pre-pregnancy preparation. Materials and methods of the study – a prospective examination of 82 patients was conducted, of which 49 women (main group) with antenatal fetal death in anamnesis and 33 women (control group) with no late reproductive losses in anamnesis. In all patients, the levels of folic acid, vitamins B_1, B_6, B_{12}, 25-hydroxyvitamin D, ferritin, Mg^{2+}, Se^{2+} and homocysteine in the blood serum were determined. The concentration of vitamins D, B_1, B_6, B_{12}, folic acid, ferritin and homocysteine was determined using enzyme-linked immunosorbent assay (Monobind (USA)). The determination of Mg^{2+} concentration was carried out using the colorimetric method (Roche Diagnostics, Switzerland). Selenium was determined using atomic absorption spectrophotometry (PerkinElmer, USA). According to the results obtained, the women in the main group had statistically significant differences in compared with the control group women: lower concentration of folic acid by 28.2%, vitamin B_6 by 24.3%; B_{12} by 23.7%, Vitamin D was 18.4 ± 1.1 ng/ml, which is 15.6% less than in the control group and 38.7% less than the lower limit of reference values. The concentration of Mg^{2+} was recorded as 1.4 times lower, as well as Se^{2+} 1.2 times in the main group. Alterations in the vitamin and mineral profile can contribute to the activation of oxidative stress, endothelial dysfunction and, as a result, placental insufficiency, which are currently considered one of the key pathogenetic links of antenatal fetal death. The obtained results emphasize the feasibility of the vitamin and mineral compounds levels determination in the pre-pregnancy preparation of women with a burdened obstetric history, which will allow the formation of individual strategies to prevent pregnancy complications and repeated perinatal losses.*

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Keywords: Ferritins, Folic Acid, Homocysteine, Pregnancy Complications, Vitamin D, Vitamin B, Antenatal Fetal Death.

Introduction

Antenatal fetal death (AFD) is one of the most severe complications of pregnancy, which is recorded in 2-4 cases per 1000 births in a high level developed countries and in 10-20 cases per 1000 births in a low level developed ones [1]. The highest proportion of antenatal fetal deaths occurs between 32 and 36 weeks of gestation. In 2021, the global rate of preterm births beyond 22 weeks was 23.0 per 1000 births, equivalent to over 3 million cases per year, of which 77% occurred in South Asia and Africa [2, 3]. In Ukraine, the incidence of AFD ranges from 4 to 8 cases per 1000 live births [4, 5]. In the last 5 years, there has been a gradual decrease in perinatal losses, but the rates of AFD remain stably high.

The etiopathogenesis of stillbirth is multifactorial and includes factors such as infectious, chromosomal abnormalities, obstetric complications, thrombophilia, immune and endocrine disorders about 30% of stillbirths remain unexplained [6]. At the current stage of research, special attention is paid to the role of endothelial dysfunction in the pathogenesis of AFD, which can serve as a marker and a factor in gestational complications. It has been established that the state of the endothelium significantly depends on the balance of vitamin and mineral compounds, which play a key role in the processes of angiogenesis, blood clotting and the formation of placental blood flow [7].

During pregnancy, the need for micronutrients increases by 20–50%, while the nutritional security of women of reproductive age often remains insufficient. In 70–80% of pregnant women, a combination of deficiencies of three or more vitamins and minerals is recorded, in particular folate, vitamins of group B, D, iron, zinc, iodine, magnesium and selenium [8, 9]. Nutrient deficiencies are associated with an increased risk of preeclampsia, fetal growth restriction, congenital malformations, preterm birth, and stillbirth [10]. It is also known that reproductive loss in anamnesis significantly increases the risks of repeated obstetric complications in subsequent pregnancies, including perinatal mortality, preterm birth, and fetal growth restriction [11].

Currently, the role of folate during pregnancy is well understood, with the main focus being on preventing neural tube defects, congenital heart defects, and fetal growth retardation [12, 13]. In case of impaired folate metabolism, the need for folates and cofactors folate metabolism (vitamins B_2, B_6, B_{12}) increases significantly, the processes of DNA repair, cell proliferation and vascular remodeling are inhibited, which is especially critical in the conditions of placenta formation [14-16]. Decreased activity of folate enzymes metabolism disrupts the transformation of homocysteine into methionine and reduces the production of S- adenosylmethionine – a universal donor of methyl groups necessary for DNA methylation and epigenetic regulation of embryonic and placental development [17].

Vitamin D also plays an important role in the proper functioning of the maternal-placental-fetal system. 25(OH)D deficiency is associated with an increased risk of placental dysfunction, preeclampsia, fetal growth retardation, and AFD [18]. Combined deficiency of folate, vitamin D, and iron, a cofactor for vitamin D activation, is found in some pregnant women, especially those with low socioeconomic status [19, 20].

The use of multivitamin complexes with prophylactic dosage increases the effectiveness of eliminating deficiencies of folate, vitamin D, iron and other nutrients during the preconception stage, which has been demonstrated in many studies and confirmed by a decrease in the frequency of preterm birth, low birth weight of the newborn and a reduced risk of AFD [21-23].

Thus, assessment of vitamin and mineral status in women with AFD in anamnesis is of significant importance in the pre-pregnancy period. Insufficiency of vital trace elements and vitamins may be a hidden factor in disorders of placentation, uteroplacental blood flow and intrauterine development of the fetus. Detection

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and timely correction of micronutrient disorders in the pre-pregnancy period opens up the possibility of personalized prevention, reducing the risk of adverse reproductive outcomes and improving perinatal outcomes [24, 25].

Aim

To determine the concentrations of vitamin and mineral compounds, namely B vitamins, folic acid, vitamin D, ferritin, magnesium, selenium, and homocysteine, in women with antenatal fetal death in anamnesis on the pre-pregnancy stage.

Materials and methods

A prospective examination of 82 women who admitted in the pre-pregnancy period to plan their next pregnancy was conducted. The main group included women with AFD in anamnesis (n=49), the control group consisted of patients with no late reproductive losses in anamnesis (n=33). The examination was conducted on the basis of the outpatient department of obstetrics and gynecological observation of the Maternity and Childhood Center of the Municipal Non-Profit Enterprise «Kyiv City Clinical Hospital №5» in the period between 2022 and 2024 years. Patients of both groups were representatively compared by age and level of social security.

Inclusion criteria: childbirth in anamnesis, voluntary consent to participate in this study.

Exclusion criteria: oncological condition; severe somatic or mental illnesses; infectious diseases in the acute stage; taking medications that affect vitamin metabolism or hormonal levels; refusal to participate at any stage of the study.

All patients were tested for the concentration of B vitamins, namely: thiamine (B_1), pyridoxine (B_6), cyanocobalamin (B_{12}), folic acid, vitamin D (25(OH)D), ferritin, magnesium (Mg^{2+}), selenium (Se^{2+}), and homocysteine in their blood serum.

Venous blood was collected on an empty stomach, from the cubital vein into two test tubes. To determine the level of vitamins D, B_1, B_6, B_{12}, folic acid, ferritin, Se^{2+} and homocysteine, gel – free tubes were used. To determine Mg^{2+}, a tube with lithium heparin was used. After blood collection, the centrifuged serum was stored at -20 °C until analysis. The concentration of vitamins D, B_1, B_6, B_{12}, folic acid, ferritin, and homocysteine was determined by enzyme-linked immunosorbent assay (ELISA) using commercial kits according to the manufacturer's protocols (Monobind (USA)). Mg^{2+} concentration was determined by the colorimetric method (Roche Diagnostics, Switzerland). Se^{2+} was determined using atomic absorption spectrophotometry (PerkinElmer, USA).

Statistica 10 software (StatSoft, USA) was used on a personal computer with Microsoft Excel 2019 software using parametric and nonparametric analysis methods. Quantitative variables were presented as mean values (M) with the corresponding standard error (m). Student's t-test was used to assess the statistical significance of intergroup differences. Fisher's exact test was used to compare categorical variables. A statistically significant difference was considered a difference at $p<0.05$.

The study was conducted in accordance with the principles of the Declaration of Helsinki. The research protocol was approved by the Bioethics Committee of the Bogomolets National Medical University (protocol No. 181 dated 01/29/2024).

Results

The average age of patients in the main group was 29.6±2.3 years; in the control group – 30.2±2.4 years ($p>0.05$). All patients of both examined groups lived in the Kyiv region. The somatic anamnesis in the main group was burdened by condition of the cardiovascular system (19 (38.8%) women), thyroid gland (13 (26.5%) women); metabolic syndrome (7 (14.3%) patients); diseases of the gastrointestinal tract and hepatobiliary system (13 (26.5%) women) urinary tract infection (13 (26.5%) women). In the control group, pathology of the cardiovascular system was noted in 8 (24.2%) women; thyroid gland – in 6 (18.1%) patients, diseases of the urinary system – in 6 (18.1%).

Among gynecological pathologies, inflammatory diseases of the urogenital tract prevailed (main group – 19 (38.8%); control group – 11 (33.3%) women); uterine leiomyoma (main group – 11 (22.4%); control group – 4 (12.1%) women, $p<0.05$); menstrual cycle disorders, manifested by dysmenorrhea (main group – 15 (30.6%); control group – 8 (24.2%) women), irregular menstrual cycle (main group – 4 (8.2%); control group – 2 (6.1%) women).

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Analyzing the concentration of folic acid in the studied groups, as a key microelement for ensuring women's health and preventing perinatal pathology, it was found that the concentration of folic acid was found to be 1.5 times lower in patients of the main group, compared with the results in the control group ($p<0.05$) (Table 1).

Table 1. The content of markers of folate metabolism in women of the examined groups (M±m)

Indicator	Survey groups	
	Main group (n=49)	Control group (n=33)
Homocysteine (μmol/L)	16.7±1, 2*	8.7±0.7
Folic acid (ng /ml)	8.9±0.6 *	12.4±0.7

Note: * - statistically significant differences compared to the control group ($p<0.05$)

It is worth noting that one of the activators of oxidative stress and endothelial dysfunction is a high level of homocysteine, which is one of the markers of impaired folate metabolism. The average value of homocysteine in patients of the main group was significantly increased and exceeded the values of the control group by 1.6 times ($p<0.05$). In particular, a third of patients of the main group (17 (34.7%) women) had significant hyperhomocysteinemia in combination with a decrease in serum folic acid.

The results obtained regarding the violation of folate metabolism necessitated the assessment of the levels of B vitamins (B_1, B_6, and B_{12}), which participate in the biosynthesis of amino acids, purine, and pyramidine bases (Table 2).

According to the data obtained, patients in the main group had a significant decrease in vitamin B_6 levels by 24.2% compared to the control group, as well as a decrease in vitamin B_{12} concentration by 23.7% ($p<0.05$), which coincided with anamnestic data on pathology of the gastrointestinal tract and hepatobiliary system in 26.5% of women in the main group.

The average value of vitamin B_1 in both study groups was within the reference values (70–180 ng/ml) and the difference in the average value of thiamine between the study groups was

Table 2. Concentration of B vitamins in women of the examined groups (M±m)

Indicator	Survey groups	
	Main group (n=49)	Control group (n=33)
Pyridoxine (B_6) (ng/ml)	56.8±4.6*	74.9±5.3
Thiamine (B_1) (ng/ml)	106.3± 8.4	112.5±9.7
Cyanocobalamin (B_{12}) (pg/ml)	147.4±6.7*	193.2±10.3

Note: * - statistically significant differences compared to the control group ($p<0.05$)

5.5% ($p>0.05$). However, vitamin B_1 deficiency was detected in the main group, 14 (28.6%) women were found to have a deficiency state in relation to this micronutrient.

Given the significant percentage of women with chronic pathology of the gastrointestinal tract and hepatobiliary system in the main group (26.5%), the level of ferritin was determined – a cofactor of cytochrome P_{450} enzymes, which is involved in the conversion of vitamin D to the biologically active form of calcitriol. In women of the main group, the average ferritin level was 14.6±1.1 ng/ml, which is 1.8 times lower than in the control group (26.3±1.6 ng/ml, $p<0.05$).

Analyzing the results of the study of the content of cofactors with pronounced antioxidant and anti-inflammatory properties (25(OH)D, Mg^{2+}, Se^{2+}), a significant difference was established between the studied groups (Table 3).

Table 3. Concentration of cofactors with antioxidant properties in women of the examined groups (M±m)

Indicator	Survey groups	
	Main group (n=49)	Control group (n=33)
Vitamin D (25(OH)D), ng/ml	18.4±1.1 *	21.8±1.3
Magnesium (Mg^{2+}), mmol/l	0.69±0.06*	0.97±0.08
Selenium (Se^{2+}), μg/l	79.1±5.9	97.3±7.2

Note: * - statistically significant differences compared to the control group ($p<0.05$)

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The average value of the concentration of 25(OH)D in women of the main group was 18.4±1.1 ng/ml, which is 15.6% lower than the similar indicator in the control group (21.8±1.6 ng/ml; $p<0.05$) and 38.7% lower than the lower limit of the reference value (30–70 ng/ml). In particular, vitamin D deficiency (<20 ng/ml) was detected in 67.3% of women of the main group versus 24.2% in the control group. A similar situation was found in the study of Mg^{2+} in the examined groups, where the concentration of Mg^{2+} was 1.4 times lower in the main group compared to the control group ($p<0.05$). Clinically, we associated the high frequency of violation of menstrual function (30.6%) in the main group with this deficiency condition.

Selenium, as the main antioxidant micronutrient, deserved no less attention, where in both examined groups it was within the reference value (70–150 μg/l). However, the concentration of Se^{2+} in the main group was lower by 18.7% compared to the control group. This was clinically confirmed by the high incidence of thyroid disease (26.5%) in women of the main group.

Thus, the obtained results of the study of vitamin and mineral compounds demonstrate significant changes in micronutrient status in women with a history of antenatal fetal death and indicate a pathogenetically combined mechanism of the occurrence of deficient states, and also justify the feasibility of including an assessment of vitamin and mineral metabolism in the pre-pregnancy examination of women with a burdened obstetric history.

Discussion.

The results of the study indicate significant deviations in the concentrations of vitamin and mineral compounds in women with a history of ADP in the pre-pregnancy stage, which is consistent with the results of recent studies. In particular, reduced levels of folic acid, vitamin B_6, B_{12} and vitamin D in reproductive losses are consistent with the results obtained in the works of Lindqvist PG (2025), which indicated a correlation between folate and B vitamin deficiency, hyperhomocysteinemia and an increased risk of thrombotic complications. At the same time, Petersen JM et.al. (2023) emphasize that the best period for correcting deficiency states, in order to prevent perinatal pathology, is the pre-pregnancy period. A systematic review showed an association between low selenium levels and pregnancy loss, preeclampsia and premature birth. In the studies of Dahlen CR (2022), lower Se^{2+} values were found in the group with pregnancy losses compared to controls. The results obtained confirm the hypothesis of the study and also have practical significance for the formation of pregravid preparation algorithms. The limitation of our study at this stage is a relatively small sample and the lack of dynamic monitoring of the course of pregnancy after correction of deficiencies, which requires further research.

Conclusions

1. The study found that women with antenatal fetal death in anamnesis have significantly lower vitamin and mineral metabolism indicators, in particular, a 1.5-fold lower level of folic acid compared to women without a history of late reproductive losses, which coincides with an increased level of average homocysteine concentration (16.7±1.2 μmol/l versus 8.7±0.7 μmol/l, $p<0.05$) and indicates high alertness to oxidative stress and, as a prognostic factor, to the development of endothelial dysfunction and prompts the mandatory correction of deficient states during the preconception stage.

2. The concentration of vitamins B_6, B_{12}, 25(OH)D was recorded in a significantly lower concentration in the main group, namely: vitamin B_6 was reduced by 24.2%; vitamin B_{12} – by 23.7%; 25(OH)D – by 15.6% compared to the control group and by 38.7% compared to the lower limit of reference values ($p<0.05$).

3. The results of the analysis of the content of micronutrients with pronounced antioxidant and anti-inflammatory effects showed a reduced amount of Mg^{2+} by 1.4 times and Se^{2+} by 1,2 times compared to the value in the control group, which coincided with the clinical manifestations, namely thyroid pathology – 26.5%; metabolic syndrome – 14.3%.

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Conflict of interest

The authors declare that they have no conflicts of interest.

AI Disclosure

No AI tools were used in the preparation of this manuscript. All authors have read and approved the final version of the manuscript.

Author Contributions (CRediT taxonomy)

Conceptualization: Hychka Nazarii (ORCID: 0000-0001-9863-6207); Ilnytska Tetiana (ORCID: 0009-0005-5922-7482)

Methodology: Beniuk Vasyl (ORCID: 0000-0002-5984-3307); Hychka Nazarii;

Software: Chebotarova Antonina (ORCID: 0000-0003-4365-2685); Oleshko Viktor (ORCID: 0000-0003-2493-2892);

Validation: Beniuk Vasyl; Chebotarova Antonina;

Formal Analysis: Hychka Nazarii; Oleshko Viktor;

Investigation: Hychka Nazarii; Chebotarova Antonina;

Resources: Beniuk Vasyl; Hychka Nazarii;

Data Curation: Hychka Nazarii;

Writing – Original Draft Preparation: Ilnytska Tetiana;

Writing – Review & Editing: Beniuk Vasyl; Oleshko Viktor;

Visualization: Chebotarova Antonina; Ilnytska Tetiana;

Supervision: Beniuk Vasyl;

Project Administration: Hychka Nazarii; Beniuk Vasyl;

Funding Acquisition: Hychka Nazarii.

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Особливості концентрації вітамінно-мінеральних сполук у жінок з антенатальною загибеллю в анамнезі на прегравідарному етапі.

Тетяна Ільницька[1], Василь Бенюк[1], Назарій Гичка[2], Віктор Олешко[1], Антоніна Чеботарьова[1]

[1] Кафедра акушерства і гінекології № 3, Національний медичний університет імені О.О. Богомольця, м. Київ, Україна

[2] Комунальне некомерційне підприємство «Київська міська клінічна лікарня №5»

Address for correspondence:

Antonina Chebotarova

E- mail: doctortonya@ukr.net

Анотація: *Антенатальна загибель плода є однією з тяжких акушерських патологій, що має значний вплив як на соматичне здоров'я, так і на психоемоційний стан жінки. В сучасних джерелах з'являються дані щодо ролі вітамінно-мінерального балансу у етіопатогенезі*

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антенатальної загибелі плода шляхом впливу на процеси ангіогенезу та формування плацентарного кровотоку. Тому, дослідження рівня вітамінно-мінеральних сполук у жінок із антенатальною загибеллю плода в анамнезі набуло особливої актуальності та спонукало до формування мети нашого дослідження: визначити особливості концентрації фолієвої кислоти, вітамінів групи В, D, феритину гомоцистеїну у жінок із антенатальною загибеллю плода в анамнезі на етапі прегравідарної підготовки. Матеріали та методи дослідження – проведено проспективне обстеження 82 пацієнток, з яких 49 жінок (основна група) з антенатальною загибеллю плода в анамнезі та 33 жінки (контрольна група), у яких відсутні пізні репродуктивні втрати в анамнезі. У всіх пацієнток визначено рівень фолієвої кислоти, вітамінів B_1, B_6, B_{12}, 25-гідроксивітаміну D, феритину, Mg^{2+}, Se^{2+} та гомоцистеїну в сироватці крові. Концентрацію вітамінів D, B_1, B_6, B_{12}, фолієвої кислоти, феритину, та гомоцистеїну визначали за допомогою імуноферментного аналізу (Monobind (США)). Визначення концентрації магнію поводили за допомогою колометричного методу (Roche Diagnostics, Швейцарія). Селен визначали за допомогою атомноабсобційної спектрофотометрії (PerkinElmer, США). Згідно отриманих результатів, у жінок основної групи виявлено статистично достовірно нижчу концентрації фолієвої кислоти в 1,5 разів , вітаміну B_6 на 24,3%; B_{12} на 23,7% відносно контрольної групи. Вітамін D становив 18,4±1,1 нг/мл, що на 15,6% менше ніж в контрольній групі та на 38,7% відносно нижньої межі референтних значень. Концентрація Mg^{2+} зареєстрована нижчою в 1,4 разів, а також Se^{2+} на 18,7% в основній групі. Порушення показників вітамінно-мінерального профілю можуть сприяти активації оксидативного стресу, ендотеліальної дисфункції та, як наслідок, плацентраної недостатності, які наразі розглядаються як одні з ключових патогенетичних ланок антенатальної загибелі плода. Отримані результати підкреслюють доцільність включення визначення рівнів вітамінно-мінеральних сполук до структури прегравідарної підготовки жінок із обтяженим акушерським анамнезом, що дозволить сформувати індивідуальні стратегії профілактики ускладнень вагітності та повторних перинатальних втрат.

Ключові слова. Антенатальна загибель плода, вітамін Д, вітаміни групи В, гомоцистеїн, ускладнення вагітності, феритин, фолієва кислота.