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Original Research

Effects of Maternal Micronutrient Levels and Homocysteine on Pregnancy Complications and Outcomes: A Prospective Cohort Study

Gul Ozel Dogan[1,*], Orhan Sahin[1]

[1]Department of Gynecology and Obstetrics, University of Health Sciences, Sisli Hamidiye Etfal Training and Research Hospital, 34098 Istanbul, Türkiye

*Correspondence: gulozeldogan@hotmail.com (Gul Ozel Dogan)

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Abstract

Background: Metabolic processes involving homocysteine and associated micronutrients (e.g., vitamin B12 and folic acid) are critical for cellular function, vascular development, and hormonal regulation during pregnancy, and they play a crucial role in both maternal and fetal health. However, evidence on the effects of micronutrient supplementation in preventing pregnancy-related complications remains limited. This prospective cohort study aims to investigate the effects of maternal micronutrient status and homocysteine levels in early pregnancy, with a focus on their potential impact on maternal and fetal health outcomes in a Turkish population. **Methods**: First-trimester blood parameters and sociodemographic data were recorded from 79 pregnant women, who were then followed until delivery. Serum levels of ferritin, vitamin D, folic acid, vitamin B12, and homocysteine were measured. Maternal and fetal complications were monitored throughout pregnancy, and any pregnancy-related adverse outcomes were documented. Participants with low micronutrient levels received supplementation. **Results**: Pregnancy-related maternal and fetal complications were as follows: gestational diabetes mellitus (21%), gestational hypertension (13%), hypothyroidism during pregnancy (17%), and preterm birth (25%). There were no significant differences in maternal blood parameters, including vitamin B12, vitamin D, folic acid, ferritin, international normalized ratio (INR), homocysteine, or hemogram levels between pregnant women with complications and those without ($p > 0.05$). **Conclusions**: Our findings suggest that neither micronutrient levels nor homocysteine alone account for pregnancy complications. However, this study underscores the potential combined impact of these factors on maternal and fetal outcomes.

Keywords: maternal nutrition; folic acid; pregnancy outcomes; micronutrient deficiency; hyperhomocysteinemia

1. Introduction

The first trimester of pregnancy is a critical period characterized by rapid fetal development and significant physiological changes in the maternal body. Key micronutrients such as vitamin D, vitamin B12, folic acid, and iron are essential for supporting both maternal health and fetal growth. Deficiencies in these nutrients during early pregnancy have been associated with a higher risk of complications in both the mother and fetus. Understanding the role of these micronutrients is crucial for developing strategies to improve maternal and fetal outcomes and to prevent potential health complications [1]. Research on the role of micronutrients in pregnancy has advanced significantly over the past two decades. Adequate levels of folate and vitamin B12 in early pregnancy have been shown to significantly reduce the risk of neural tube defects and other congenital malformations [2]. Elevated homocysteine levels, by contrast, have been associated with increased risks of miscarriage, preeclampsia, and low birth weight [3]. Ferritin levels are indicative of maternal iron stores, and maintaining optimal iron levels is essential for preventing anemia, which is associated with preterm birth and low fetal weight [4]. Vitamin D, by contrast, plays a vital role in bone formation and immune regulation, with deficiency linked to gestational diabetes and fetal growth restriction [5]. Despite their importance, the interplay between these factors and their effects on pregnancy outcomes remain incompletely understood.

Micronutrient deficiencies can potentially impact long-term outcomes in the fetus, including survival, cognitive development, and cardiometabolic health, highlighting their importance during early life [6]. Existing studies highlight the adverse effects of micronutrient deficiencies and hyperhomocysteinemia on pregnancy outcomes; however, significant knowledge gaps remain [1,3]. Although some studies have established associations between low micronutrient levels and pregnancy complications, findings are frequently inconsistent or limited by small sample sizes, variability in supplementation protocols, and insufficient long-term follow-up [7,8]. Poor pregnancy outcomes, including preterm birth, intrauterine growth restriction (IUGR), and miscarriage, have been closely linked to alterations in maternal nutritional status during pregnancy [7]. Conversely, a Cochrane meta-analysis on vitamin supplementation for miscarriage prevention concluded that there is currently insufficient evidence to determine the effects of various vitamin combinations on miscarriage and related outcomes [8]. It is evident that adequate evidence linking nutritional de-

ficiencies in the first trimester to pregnancy outcomes has yet to be established. Furthermore, few studies comprehensively evaluate the combined effects of micronutrient levels and homocysteine on both maternal and fetal outcomes, particularly in populations with distinct nutritional and cultural profiles, such as those in Türkiye.

In a previous study, we measured ferritin, vitamin D, folic acid, and vitamin B12 levels in women who experienced first-trimester miscarriages compared to those with healthy pregnancies. The results showed significantly lower micronutrient levels in women who experienced miscarriage, with ferritin emerging as a potential protective factor against pregnancy loss [9]. Micronutrient levels during early fetal development influence hormonal metabolism, vascular growth, and organogenesis, thereby increasing the risk of cardiometabolic issues like obesity, type 2 diabetes, and cardiovascular diseases later in life [10]. Limited research exists on correcting maternal micronutrient deficiencies through supplementation, highlighting the need for further studies on these interventions. To address these questions, participants in the control group of our aforementioned study, whose blood analyses were conducted during the first trimester, were followed until the end of their pregnancy. Maternal and fetal well-being were closely monitored throughout the study period. Pregnant women with serum micronutrient and homocysteine levels outside normal ranges received supplement treatment, such as vitamin D, Vitamin B12, folic acid, and iron preparations. They were then compared to those with normal micronutrient levels to explore the development of pregnancy complications. By analyzing the associations between early nutritional status and outcomes, this research aimed to contribute to the refinement of clinical guidelines and prenatal care strategies.

2. Methods

This observational cohort study involved the prospective follow-up of pregnant women. The study was approved by the Ethics Committee of the University of Health Sciences, Sisli Hamidiye Etfal Training and Research Hospital, Istanbul, Türkiye (Ethics approval number: 2024-4479), and was performed in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants. Pregnant women attending the obstetric outpatient clinic between January 1, 2023, and January 1, 2024, were enrolled in the study. Demographic characteristics, chronic diseases, and blood parameters of the participants were recorded.

2.1 Participants

Previously, we compared the blood analyses from 88 women who experienced first-trimester miscarriage with those from 88 healthy pregnant women in the control group [6]. In this study, we aimed to follow the medical status of the 88 healthy control participants from the previous study throughout pregnancy, up until delivery. However, 9 women discontinued follow-up at our clinic, making their data unavailable for analysis. As a result, the medical records of 79 participants were reviewed and included in the final evaluation. All participants were between 18 and 40 years of age, had singleton pregnancies, and had not received any prophylactic nutritional supplements at the time of their initial assessment.

Data on participants' clinical and sociodemographic characteristics, as well as first-trimester serum levels of ferritin, 25-hydroxyvitamin D (25(OH) vitamin D), vitamin B12, folic acid, and homocysteine, along with complete blood count, blood type, and coagulation parameters were recorded in a database prepared by the researchers. Participants were questioned about their age, smoking status, weight, height, alcohol consumption, consanguineous marriage, gravidity, parity, last menstrual period, history of abortion, and presence of chronic diseases; this information was recorded in case report forms. Gestational age was calculated and recorded based on the date of the last menstrual date. All participants underwent ultrasonographic examination using a Siemens ACUSON X300 ultrasound system (software version 5.6.0, lot number: USX300-2021-07; Siemens Medical Solutions USA, Inc., Mountain View, CA, USA), conducted by the same physician. The date of the last menstrual period and the ultrasonographic examination were recorded at the time of recruitment to determine gestational age. The medical records of participants were documented from the first trimester through delivery.

2.1.1 Inclusion Criteria

- Pregnant women aged 18–40 years and between 5 and 14 gestational weeks.
- Women with documented medical histories and available serum levels of vitamin B12, folic acid, homocysteine, ferritin, and 25(OH) vitamin D.

2.1.2 Exclusion Criteria

- Pregnant women under 18 years of age and over 40 years.
- Women with anatomical or genetic abnormalities, such as septate uterus, bicornuate uterus, or other major malformations, as well as those with autoimmune diseases, endocrine disorders (including diabetes mellitus, thyroid disorders [e.g., hypothyroidism or hyperthyroidism], adrenal gland disorders, and polycystic ovary syndrome [PCOS]), or chronic conditions related to high blood pressure.
- Women with multiple pregnancies.

Participants were then divided into two groups based on the presence or absence of maternal or fetal health complications during pregnancy. The clinical characteristics and blood parameters of the group that developed complications were compared to those of women with healthy pregnancies. Factors contributing to the development of mater-

nal and fetal complications were also investigated. Cases with micronutrient deficiencies received supplementation treatment. Dosages and combinations were administered according to the guidelines established by the Turkish Ministry of Health for pregnant women [11]. Specifically, daily supplementation included elemental iron (27–60 mg), folic acid (400–800 µg), vitamin D (600–1000 IU), and vitamin B12 (250–500 µg). The duration of supplementation varied according to the severity of the deficiency and continued until optimal levels were achieved or until delivery.

2.2 Collection of Samples

After an 8-hour fast, venous blood samples were collected into vacuum gel tubes to assess the hemogram and international normalized ratio (INR) in all participants. Total plasma homocysteine was measured using an enzymatic cycling assay on the Cobas 8000 analyzer (software version 3.0.0, lot number: 123456; Roche Diagnostics GmbH, Mannheim, Germany) with a reference range of 5–15 µmol/L. Vitamin B12, folic acid, 25(OH) vitamin D, and ferritin levels were measured via chemiluminescence immunoassay on the same Cobas 8000 system (software version 4.1.2, lot number: 789012; Roche Diagnostics GmbH, Mannheim, Germany). The reference ranges were 180–771 ng/L for vitamin B12, 3.89–26.8 µg/L for folic acid, 20 µg/L for 25(OH) vitamin D, and 13–150 µg/L for ferritin.

2.3 Maternal Data Collection

Data were collected through a researcher-designed questionnaire, physical examinations, and laboratory investigations. A structured, preformatted questionnaire was used at the baseline visit to collect information on age, weight, height, and medical history of chronic diseases (including diabetes, hypertension, coronary artery disease, and stroke), history of addiction, nutritional status, and pregnancy-related history. Postpartum, a retrospective review was conducted to document patients' pregnancy diagnoses, supplement use, delivery complications, and mode of birth (vaginal delivery or caesarean section). All relevant data were collected and recorded based on their pregnancy follow-up records. Body mass index (BMI) was calculated as weight divided by height squared (kg/m^2). Participants were monitored from early pregnancy until delivery through physical examinations, routine laboratory tests, and ultrasound evaluations to assess maternal and fetal health. Specific conditions evaluated included gestational diabetes mellitus, gestational hypertension, hypothyroidism during pregnancy, IUGR, microcephaly, oligohydramnios, preeclampsia, preterm birth, Rhesus (Rh) factor incompatibility, and wound infection. In accordance with the Turkish Ministry of Health protocol [11], routine laboratory tests were reviewed for every pregnant woman attending our hospital at any point during the first trimester. If folic acid levels are normal, a daily oral dose of 400 micrograms of folic acid is prescribed throughout the first trimester. During the second and third trimesters, multivitamin tablets containing 400 micrograms of folic acid along with other vitamins were administered. For cases with abnormal results at the start of each trimester, follow-up blood tests were conducted. Pregnancy check-ups were conducted monthly until the 32nd week, every two weeks from the 32nd to the 36th week, and weekly from the 36th week until delivery.

2.4 Newborn Data Collection

Neonatal characteristics, including birth weight, presence of IUGR, preterm birth, gestational age at birth, and neonatal death within 7 days postpartum, were recorded at the time of delivery (n = 79).

2.5 Statistical Analysis

For the statistical analysis, IBM SPSS Statistics for Windows, Version 21.0 (IBM Corp., Armonk, NY, USA) was used. Descriptive statistics were presented as numbers and percentages for categorical variables, and as means $\pm$ standard deviations (SD), medians (min-max), or medians (p25, p75) for continuous variables, depending on data distribution. The distribution of continuous variables was assessed using the Shapiro-Wilk test, and the homogeneity of variances was evaluated with Levene's test. Based on these assumptions, the independent samples *t*-test or Welch's *t*-test was used for normally distributed variables, while the Mann-Whitney U test was applied for non-normally distributed variables. Categorical variables were compared using the chi-square test. A *p*-value of <0.05 was considered statistically significant.

3. Results

The study included 79 participants, with a mean age of 31.9 years and an average BMI of 26.3 kg/m^2. The median values for gravida, parity, and history of abortion were 2 (range: 1–3), 1 (range: 0–2), and 0 (range: 0–0), respectively. Most participants exhibited normal coagulation parameters, ferritin levels, hemogram results, and homocysteine concentrations. However, vitamin D levels were consistently low, with mean values indicative of deficiency. Table 1 provides detailed demographic and laboratory characteristics of the participants.

A total of 79 participants were included in the study, of whom 24 (30%) experienced complications, while 55 (70%) had no complications. The median age of participants was 31.0 years in both groups ($p = 0.46$). There was no statistically significant difference in BMI between those with complications (median: 26.0) and those without complications (median: 26.0) ($p = 0.87$). However, a statistically significant difference was observed in gestational age at delivery, with participants who experienced complications having a lower median gestational age at delivery of 37.0 weeks, compared to 38.0 weeks in those without complications ($p < 0.01$). Similarly, the median newborn length

Table 1. Demographic and clinical characteristics of all participants.

Characteristics	Mean ± SD	Minimum	Maximum
Age (years)	31.9 ± 5.62	22.00	47.00
Baby Height (cm)	48.8 ± 3.59	28.00	55.00
Baby Weight (g)	3145.0 ± 559.00	900.00	4220.00
BMI (kg/m²)	26.3 ± 2.95	22.00	35.00
Last Menstrual Period (weeks)	11.9 ± 1.09	6.00	13.00
Gestational Age at Delivery (weeks)	38.0 ± 1.95	27.00	41.00
Coagulation Parameters (INR)	1.0 ± 0.06	0.90	1.18
Ferritin (µg/L)	40.5 ± 36.70	5.50	259.00
Hemoglobin (g/dL)	11.9 ± 0.87	8.10	13.40
Folic Acid (ng/mL)	14.9 ± 5.49	2.93	20.00
Homocysteine (µmol/L)	6.0 ± 1.40	3.10	10.30
Vitamin B12 (pg/mL)	298.0 ± 115.00	100.00	741.00
25(OH) Vitamin D (ng/mL)	12.1 ± 6.94	3.00	30.50

SD, standard deviation; BMI, body mass index; INR, international normalized ratio; 25(OH) Vitamin D, 25-hydroxyvitamin D.

was lower in the complication group (48.0 cm) compared to the non-complication group (49.0 cm) ($p = 0.046$). Newborn weight also differed significantly between the groups, with a mean weight of 2800 grams in the complication group compared to 3295 grams in the non-complications group ($p < 0.01$). The median gestational age based on the last menstrual period was similar between both groups, at 12.0 weeks ($p = 0.45$). The median gravida was comparable between both groups, with both having a median of 2.00 ($p = 0.74$). Parity showed no statistically significant difference between groups, with a median of 1.00 in the women without complications group and 0.50 in those with complications ($p = 0.85$). Regarding delivery types, 65% of participants without complications had a normal vaginal delivery, compared to 46% in the complication group ($p = 0.10$). Cesarean section was more common in the complication group (54%) than in the non-complication group (35%).

In terms of specific complications, gestational diabetes mellitus was observed in 5 participants (21%). Similarly, gestational hypertension (13%), hypothyroidism during pregnancy (17%), IUGR (13%), microcephaly (4.2%), oligohydramnios (4.2%), preeclampsia (13%), and preterm birth (25%) were observed. The participants developed hypothyroidism later during pregnancy, classified as new-onset gestational hypothyroidism. New-onset gestational hypothyroidism, diabetes mellitus, and hypertension were identified through routine follow-up tests conducted during antenatal care. There were no significant differences in the rates of Rh incompatibility between the groups (18% in the non-complication group vs. 8.3% in the complication group). Wound infection was observed in 3 participants (13%) within the complication group. Table 2 presents the univariable analysis comparing participants with and without complications, highlighting key differences in clinical characteristics and pregnancy outcomes between the two groups.

Table 3 presents the comparison of maternal blood analysis results between participants with and without pregnancy complications. The mean 25(OH) vitamin D levels were 12.1 µg/L in participants without complications and 9.00 µg/L in those with complications ($p = 0.52$). The majority of participants in both groups had low vitamin D levels (<20 µg/L), with 87% in the non-complication group and 92% in the complication group falling below this threshold ($p = 0.71$). The mean vitamin B12 levels were 276 ng/L in the non-complication group and 308 ng/L in the complication group ($p = 0.89$). The mean folic acid levels were 16.7 µg/L in the non-complication group and 19.3 µg/L in the complication group ($p = 0.32$). Ferritin levels were similar between the groups, with mean values of 34.0 µg/L in the non-complication group and 33.7 µg/L in the complication group ($p = 0.68$). The mean INR was 1.00 in both groups ($p = 0.34$). Mean homocysteine levels were 5.93 µmol/L in the non-complication group and 6.22 µmol/L in the complication group ($p = 0.45$). Hemogram levels were similar between the groups, with means of 12.1 g/dL and 11.9 g/dL for non-complication and complication groups, respectively ($p = 0.47$).

4. Discussion

The objective of this study was to explore the impact of maternal serum micronutrient and homocysteine levels during early gestation on pregnancy and perinatal outcomes, as well as subsequent infant development and pregnancy complications. Deficiencies in these essential nutrients during pregnancy can potentially increase risk of cardiometabolic complications by influencing hormonal metabolism, blood vessel formation, and organ development in both the mother and fetus [1]. Despite this, research on the effectiveness of micronutrient supplementation in preventing pregnancy-related complications remains limited. Our findings revealed no significant associations

Table 2. Univariable analysis of maternal and fetal outcomes based on complications.

Characteristics	Participants without complications (n = 55)	Participants with complications (n = 24)	*p*-value
Age, median [P25–75] (years)	31.0 [27.5; 35.0]	31.0 [27.8; 37.0]	0.46*
BMI, median [P25–75] (kg/m^2)	26.0 [24.0; 27.5]	26.0 [24.1; 27.2]	0.87*
Gestational Age, median [P25–75] (weeks)	38.0 [38.0; 39.0]	37.0 [36.0; 39.0]	<0.01*
Baby Height, median [P25–75] (cm)	49.0 [48.5; 50.5]	48.0 [47.0; 50.2]	0.046*
Baby Weight, mean ± SD (g)	3295 ± 318	2800 ± 802	<0.01**
Last Menstrual Period, median [P25–75] (weeks)	12.0 [10.0; 14.0]	12.0 [9.5; 14.5]	0.45*
Gravida, median [P25–75]	2.00 [1; 3]	2.00 [1; 4]	0.74*
Parity, median [P25–75]	1.00 [0; 3]	0.50 [1; 3]	0.85*
Delivery Types	-	-	0.10***
Vaginal Delivery	36 (65%)	11 (46%)	-
Cesarean Section	19 (35%)	13 (54%)	-
Rh Incompatibility, n (%)	10 (18%)	2 (8.3%)	0.328***
Gestational Diabetes Mellitus (n) (%)	0 (0%)	5 (21%)	-
Gestational Hypertension (n) (%)	0 (0%)	3 (13%)	-
Hypothyroidism During Pregnancy (n) (%)	0 (0%)	4 (17%)	-
IUGR (n) (%)	0 (0%)	3 (13%)	-
Microcephaly (n) (%)	0 (0%)	1 (4.2%)	-
Oligohydramnios (n) (%)	0 (0%)	1 (4.2%)	-
Preeclampsia (n) (%)	0 (0%)	3 (13%)	-
Preterm Birth (n) (%)	0 (0%)	6 (25%)	-
Rh Incompatibility (n) (%)	10 (18%)	2 (8.3%)	-
Wound Infection (n) (%)	0 (0%)	3 (13%)	-

Statistical tests used: * Mann-Whitney U test; ** Welch's *t*-test; *** Chi-square test (χ^2). BMI, body mass index; Rh, Rhesus; IUGR, intrauterine growth restriction.

between first-trimester micronutrient deficiencies, including vitamin D, folic acid, vitamin B12, ferritin, or homocysteine, and the development of pregnancy-related complications.

In our study, most pregnancies without complications showed low micronutrient levels: 87% had low 25(OH) vitamin D, 9.1% had low vitamin B12, and 7.3% had low ferritin. Among pregnancies with complications, 92% had low 25(OH) vitamin D, 13% had low vitamin B12, and 8.3% had low ferritin. This similarity in micronutrient deficiencies between the two groups may help explain the lack of significant associations observed. Compared to previous studies, variations in sample size, population characteristics, and supplementation protocols may have influenced the observed outcomes. For example, earlier research has often reported stronger links between low micronutrient levels and pregnancy complications, especially in groups with more severe deficiencies or differing follow-up methodologies [12,13]. In a study by Xie *et al.* [12], the researchers explored the causal links between specific maternal micronutrient levels and the occurrence of pregnancy complications. The research highlighted the importance of maintaining adequate levels of these micronutrients to reduce the risk of pregnancy complications. Their analysis found evidence that higher circulating levels of vitamin E may help protect against the risk of spontaneous abortion. Additionally, a genetic predisposition to higher vitamin B12 levels was associated with a lower risk of developing spina bifida. On the other hand, no association was reported between folate levels and the risk of spontaneous abortion or preterm birth. Consistent with our findings, no causal relationships were identified between other micronutrients and conditions like gestational diabetes mellitus and gestational hypertension. In our study, early supplementation based on clinical guidelines may have mitigated the potential impact of these deficiencies, thereby reducing the likelihood of detecting significant associations. Additionally, the sample size may have been insufficient to detect subtle differences. These factors should be considered when interpreting our findings and designing future research.

Women who experienced first-trimester miscarriages had lower levels of ferritin, vitamin D, folic acid, and vitamin B12 compared to those with healthy pregnancies, with ferritin identified as a potential protective factor [9]. Building on these findings, our study examined whether first-trimester deficiencies in micronutrients, such as folic acid, ferritin, homocysteine, vitamin D, and vitamin B12, were associated with complications like gestational diabetes, hypertension, hypothyroidism, preterm birth, and adverse neonatal outcomes. Women with abnormal micronutrient levels received supplementation and were monitored

Table 3. Comparison of maternal blood analysis results between participants with and without pregnancy complications.

Maternal Blood Analysis (First Trimester)	Participants without Complications (n = 55)	Participants with Complications (n = 24)	*p*-value
25(OH) Vitamin D, median (min–max) [µg/L]	12.1 (6.54–16.4)	9.00 (7.56–13.3)	0.52**
25(OH) Vitamin D, n (%)			0.71‡
Low (<20 µg/L)	48 (87%)	22 (92%)	
Normal (>20 µg/L)	7 (13%)	2 (8.3%)	
Vitamin B12, median (min–max) [ng/L]	276 (231–352)	308 (195–362)	0.89**
Vitamin B12, n (%)			0.69‡
Normal (180–771 ng/L)	50 (91%)	21 (88%)	
Low (<180 ng/L)	5 (9.1%)	3 (13%)	
Folic Acid, median (min–max) [µg/L]	16.7 (10.3–20.0)	19.3 (10.1–20.0)	0.32*
Folic Acid, n (%)			0.21‡
Normal (3.89–26.8 µg/L)	39 (71%)	14 (58%)	
High (>26.8 µg/L)	16 (29%)	9 (38%)	
Low (<3.89 µg/L)	0 (0%)	1 (4.2%)	
Ferritin, median (min–max) [µg/L]	34.0 (16.9–50.4)	33.7 (14.9–46.0)	0.68**
Ferritin, n (%)			1.00‡
Normal (13–150 µg/L)	51 (93%)	22 (92%)	
Low (<13 µg/L)	4 (7.3%)	2 (8.3%)	
INR, median (min–max)	1.00 (0.99–1.08)	1.00 (0.975–1.05)	0.34*
Homocysteine, mean ± SD [µmol/L]	5.93 ± 1.30	6.22 ± 1.61	0.45**
Hemogram, median (min–max) [g/L]	12.1 (11.4–12.5)	11.9 (11.4–12.3)	0.47*
Hemogram, n (%)			0.86‡
Normal (11.5–15.5 g/L)	40 (73%)	17 (71%)	
Low (<11.5 g/L)	15 (27%)	7 (29%)	

Statistical tests used: * Mann-Whitney U test, ** Welch's *t*-test, ‡ Fisher's Exact test. INR, international normalized ratio; SD, standard deviation.

throughout pregnancy. No significant relationship was found between micronutrient deficiencies and pregnancy complications, suggesting that the impact of micronutrient and homocysteine serum levels may be indirect or influenced by other unmeasured factors.

Our analysis suggested that serum micronutrient and homocysteine levels alone may not directly contribute to complications, possibly due to the small sample size or other unmeasured factors. Anemia, associated with various pregnancy complications, is influenced by ferritin levels, which reflect iron storage and play a critical in fetal and placental development [10,14]. The interactions between iron, folic acid, vitamin D, and B12 may underlie pregnancy loss, supporting the importance of supplementation. The lack of differences in outcomes between women with low ferritin levels who received supplementation and those with normal levels in this study highlights the importance of nutritional interventions during pregnancy.

Homocysteine, a metabolite of methionine, is influenced by factors such as genetic mutations, deficiencies in folic acid and vitamins B6 and B12, hypothyroidism, medications, aging, and kidney dysfunction [15]. Elevated homocysteine levels (hyperhomocysteinemia) have been associated with various pregnancy complications, including recurrent pregnancy loss, preeclampsia, preterm birth, placental abruption, fetal growth restriction, and gestational diabetes [1,3,14,16]. Consequently, further research is needed to clarify the relationship between serum homocysteine levels and placental complications in pregnant women. It has been reported that lower levels of folic acid and vitamin B12 are associated with miscarriage, highlighting the importance of these micronutrients in supporting pregnancy viability [17]. Moreover, during pregnancy, homocysteine levels are typically lower due to hemodilution and increased glomerular filtration [3]. In our study, homocysteine levels were detected within the normal range, with no significant relationship observed between homocysteine levels and pregnancy complications. This finding may be attributed to hemodilution or to supplementation provided for folic acid and vitamin B12 deficiencies.

Vitamin D deficiency, commonly linked to maternal and neonatal bone disorders, is also frequently observed in women experiencing significant reproductive and obstetric complications, including preeclampsia, gestational diabetes, and preterm birth [1]. Vitamin D deficiency is prevalent among pregnant women and their newborns in Türkiye, even during the spring season, largely due to maternal lifestyle and dietary habits [18]. These factors may help explain the consistently low vitamin D levels observed in all participants in this study. Vitamin D potentially

contributes to key biological processes involved in fetal growth, supporting healthy fetal development [19]. Vitamin D supplementation has been shown to reduce the risks of low birth weight, being small for gestational age, and stillborn [20]. In the current study, consistently low vitamin D levels were observed in women during the first trimester; however, no significant association with pregnancy complications was identified, underscoring the importance of supplementation.

The current study has several limitations. As the study was conducted at a single healthcare center, its findings may not be generalizable to broader populations. The Turkish Ministry of Health recommends that the initial blood tests include screening for maternal blood counts, kidney and liver function, blood glucose levels, thyroid hormone levels, infections such as toxoplasmosis and rubella, as well as sexually transmitted diseases [21]. In the current study, participants with micronutrient deficiencies received appropriate nutritional supplementation. It is possible that the outcome of the study might have differed if supplementation had not been provided; however, withholding such treatment would not have been ethically acceptable. The primary objective of this study was not to evaluate the effects of supplementation. Nonetheless, future research on this topic would greatly enhance our understanding. Despite these limitations, our findings underscore the importance of routine nutritional screening and appropriate supplement use as integral components of prenatal care.

5. Conclusions

Our findings demonstrated that first-trimester levels of vitamin D, folic acid, vitamin B12, ferritin, and homocysteine were not significantly associated with the occurrence of pregnancy complications. However, these results suggest that supplementation may be effective in mitigating potential risks. These findings underscore the importance of routine assessment for micronutrient deficiencies and the level of homocysteine during the first trimester of pregnancy. Future studies should focus on multicenter cohorts to validate these findings and explore the potential regional or genetic factors influencing micronutrient and homocysteine levels during pregnancy.

Availability of Data and Materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Author Contributions

GOD and OS designed the research study. GOD performed the data curation and the research. GOD analyzed the data. Both authors contributed to editorial changes in the manuscript. Both authors read and approved the final manuscript. Both authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of the University of Health Sciences, Sisli Etfal Training and Research Hospital, Istanbul, Türkiye (Study Protocol Number: 2024-4479). The verbal and written consents were given by all of the participants.

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Conflict of Interest

The authors declare no conflict of interest.

Declaration of AI and AI-Assisted Technologies in the Writing Process

During the preparation of this work the authors used ChatGpt-3.5 in order to check spell and grammar. After using this tool, the authors reviewed and edited the content as needed and takes full responsibility for the content of the publication.

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