Van Med J 31 (4):275-281, 2024
DOI: 10.5505/vmj.2024. 52724

Investigation of the Relationship Between Serum Tetrahydrobiopterin and Folic Acid Levels with Preeclampsia in the Second and Third Trimesters

Mücahit Ahmet Aranlı[1], Kazım Uçkan[1], Abdülaziz Gül[1], Yusuf Başkıran[2], İzzet Çeleğen[3]

[1]Van Yüzüncü Yıl University Faculty of Medicine, Department of Gynecology and Obstetrics, Van, Türkiye
[2]Istinye University, Faculty of Medicine, Department of Gynecology and Obstetrics, Istanbul, Türkiye
[3]Van Yüzüncü Yil University, Faculty of Medicine, Department of Public Health, Van, Türkiye

Abstract

Introduction: This study investigates the relationship between serum tetrahydrobiopterin (BH4) and folic acid levels in pregnant women during the second and third trimesters and the development of preeclampsia.

Materials and Methods: This case-control study was conducted from June 2022 to February 2024 with 160 pregnant women with and without preeclampsia (PE). Participants were divided into PE and control groups and further categorized by folic acid usage into four groups: non-PE without folic acid, non-PE with folic acid, PE without folic acid, and PE with folic acid. Groups were compared in terms of demographic characteristics and various laboratory parameters.

Results: The PE group had significantly higher blood pressure, lower albumin and total protein levels, and higher urinary protein levels than the control group (P=0.001). BH4 levels were significantly lower in the PE group (P=0.001). The group without folic acid supplementation had significantly lower folate and haemoglobin levels than those with folic acid supplementation (P=0.001).

Conclusion: There is a significant association between serum BH4 and folic acid levels and the development of preeclampsia. Lower BH4 levels were observed in preeclamptic pregnant women, while folic acid supplementation positively influenced folate and haemoglobin levels. BH4 and folic acid may play a role in the pathogenesis of preeclampsia and could be potential biomarkers for assessing preeclampsia risk.

Key words: Pregnancy; preeclampsia; tetrahydrobiopterin; folic acid.

Introduction

Preeclampsia (PE) is a major hypertensive complication of pregnancy, characterised by widespread endothelial dysfunction. This condition is often linked to the failure of endovascular trophoblast invasion into maternal spiral arteries. Affecting 2-8% of pregnant women globally, PE remains one of the leading causes of maternal death (1). In PE cases, the insufficient invasion of trophoblasts can lead to the thickening of spiral arteries, increased non-invasive trophoblasts, and thrombosis in surrounding tissues. This situation can activate the coagulation system, leading to abnormal hemostasis and the formation of atheromatous plaques. Consequently, uteroplacental blood flow is compromised, resulting in fetoplacental hypoxia. This lack of proper blood flow causes an imbalance in the release and metabolism of endothelin and nitric oxide (NO) from the placenta and extraplacental tissues. This contributes to vasoconstriction, endothelial cell damage, and liver damage associated with hypertension (2). Folic acid, also known as "Pteroylmonoglutamic acid," is a water-soluble crystalline derivative with a molecular weight of 550 daltons. It serves as a coenzyme in single carbon transfer reactions. In its active form, tetrahydrofolic acid (THF), folic acid supports the biosynthesis of serine, methionine, glycine, choline, and purine nucleotides, essential for cellular metabolism and overall health (3). Low levels of folic acid and vitamin B12 are linked to elevated homocysteine levels, a sulfur-containing amino acid involved in important metabolic pathways. Adequate intake of folic acid and vitamin B12 is crucial, especially during pregnancy, as deficiencies are associated with an increased risk of cardiovascular diseases (4).

This study investigates the relationship between serum tetrahydrobiopterin (BH4) and folic acid levels and the development of preeclampsia in

*Corresponding Author Mücahit Ahmet Aranlı Van Yüzüncü Yıl University Faculty of Medicine, Department of Gynecology and Obstetrics, Van, Türkiy **E -mail:** mucahitaranli@gmail.com **Orcid:** Mücahit Ahmet Aranlı 0009-0006-8501-7136, Kazım Uçkan 0000-0002-5576-6789, Abdülaziz Gül 0000-0003-1158-5019, Yusuf Başkıran 0000-0003-1123-6062, İzzet Çeleğen 0000-0002-2749-953X

Received: 13.06.2024, Accepted: 03.10.2024

pregnant women during the second and third trimesters.

Materials and Methods

This case-control study was conducted from June 1, 2022, to February 28, 2024. The study included 80 patients diagnosed with preeclampsia (PE) according to the 2013 guidelines of the American College of Obstetricians and Gynecologists (ACOG), who presented to an Obstetrics and Gynecology Clinic at a Van Yuzuncu Yil University Medical School Hospital between 20-37 weeks of gestation. Additionally, 80 healthy pregnant women who presented within the same gestational weeks were included as the control group (8). The sample size was selected based on previous studies examining similar results in preeclampsia research. Due to the lack of preliminary data, no sample calculation was made before starting the study, and based on the current literatüre (9), a sample size of 160 cases (80 in each group) was considered sufficient to detect significant differences between the groups. However, after the completion of the study, a post hoc power analysis was performed to test the adequacy of the study power. According to the power analysis results, the power for the comparison of the main hypothesis of the study, BH4, Biopterin, NO and Folate parameters, was found to be over 99.9%. Approval was obtained from a university's Non-Interventional Clinical Research Ethics Committee to conduct the research. Informed consent was obtained from patients who participated in the study voluntarily and agreed to the use of their information. The inclusion criteria for the study consisted of pregnant women aged 18-35, who were multiparous, without any chronic diseases, and carrying singleton pregnancies. Exclusion criteria included a history of chronic conditions such as diabetes, autoimmune diseases, chronic hypertension, vascular connective tissue diseases, nephropathy, rheumatic diseases, and thrombophilias. Additionally, women with multiple pregnancies, nulliparous pregnancies, a body mass index (BMI) over 30, or a personal or family history of preeclampsia were excluded from the study. Obstetric anamnesis information, demographic information, obstetric ultrasonography biometric measurements, routine tests, as well as 24-hour urine protein, serum albumin, total protein, homocysteine, vitamin B12, folate, biopterin, BH4, and NO tests were performed on the patients. Systolic and diastolic blood pressures were measured, and pregnant women meeting the PE diagnostic criteria according to the 2013 ACOG guidelines were identified. The group diagnosed with PE and the control group without PE were asked if they regularly took a 400 mcg methyl folate preparation daily in the first trimester, and the patients were divided into four groups accordingly.

Group 1: Healthy pregnant women without any chronic diseases who did not take a daily 400 mcg methyl folate preparation regularly in the first trimester.

Group 2: Healthy pregnant women without any chronic diseases who took a daily 400 mcg methyl folate preparation regularly in the first trimester.

Group 3: Pregnant women diagnosed with PE according to the 2013 ACOG guidelines did not take a daily 400 mcg methyl folate preparation regularly in the first trimester.

Group 4: Pregnant women diagnosed with PE according to the 2013 ACOG guidelines took a daily 400 mcg methyl folate preparation regularly in the first trimester.

A total of 80 cases without PE were classified 1 and 2, and a total of 80 cases diagnosed with PE were classified 3 and 4. When the total number of 160 cases was reached in both groups, no new cases were included in the study. From each patient who volunteered to participate in the study, 5 ml of venous blood was collected in a biochemistry tube, centrifuged at 4000 rpm for 10 minutes, and stored in two Eppendorf tubes at -50 degrees Celsius. The blood samples from the pregnant women were analyzed in the biochemistry laboratory using Enzyme-linked Immunosorbent Assay Kits for BH4 (Catalog No: CEG421Ge), General Biopterin ELISA Kit (Catalog No: E2158Ge), and Human Nitric Oxide ELISA Kit (Catalog No: SL1275Hu), according to the manufacturer's instructions, with a Biotek ELX800 ELISA reader.

Ethical approval: Ethical permission was obtained from the Clinical Research Ethics Committee of Van Yüzüncü Yıl University Faculty of Medicine with c
27.03.2024.

Statistical analysis: All statistical analyses were performed using version 25 of the Statistical Package for the Social Sciences (SPSS) software (SPSS Inc., Chicago, USA). The data included in the study were presented as mean and standard deviation (± SD) for the groups. The normality of the data distribution was evaluated with the Kolmogorov-Smirnov test. Differences between two groups for normally distributed data were analyzed using Student's t-test, and differences among three or more groups were analyzed using ANOVA. For non-normally distributed data,

Table 1: Comparison of Demographic and Laboratory Characteristics Between Control and PE Groups

Characteristic	Control (n=80) Mean ± S.D.	PE (n=80) Mean ± S.D.	p-value
Age (years)	29 ± 5	30 ± 5	0.283b
Gestational Age (weeks)	29 ± 5	31 ± 4	0.138b
Gravida	4 ± 2	4 ± 2	0.295b
BMI (kg/m²)	26 ± 2	27 ± 1	0.001b
SBP (mmHg)	120 ± 7	149 ± 5	0.001b
DBP (mmHg)	78 ± 4	91 ± 5	0.001b
Haemoglobin (g/dL)	12.2 ± 1.2	11.9 ± 1.3	0.085a
PLT (x10³/μL)	233 ± 60	227 ± 58	0.530b
AST (U/L)	19 ± 7	22 ± 14	0.343b
ALT (U/L)	14 ± 8	16 ± 15	0.914b
Creatinine (mg/dL)	0.57 ± 0.06	0.61 ± 0.12	0.070b
Homocysteine (μmol/L)	7.41 ± 4.82	8.51 ± 5.13	0.174b
Albumin (g/dL)	31 ± 3	28 ± 4	0.001b
Total Protein (mg/dL)	65 ± 5	62 ± 6	0.001b
Urinary Protein (mg/L)	138 ± 47	1719 ± 3565	0.001b
BH4 (pg/mL)	81.4 ± 10	67.8 ± 8.9	0.001a
Biopterin (nmol/L)	177.4 ± 15.7	186.4 ± 12.7	0.002b
NO (μmol/L)	15.2 ± 6.7	29.4 ± 21.4	0.001b
Folate (ng/mL)	9.3 ± 4.6	9.4 ± 4.5	0.802b
Vitamin B12 (pg/mL)	181 ± 69	187 ± 87	0.811b

Abbreviations: BMI: Body mass index, **SBP:** Systolic blood pressure, **DBP:** Diastolic blood pressure, **PLT:** Platelet count, **AST:** Aspartate Aminotransferase, **ALT:** Alanine Aminotransferase, **BH4:** Tetrahydrobiopterin, **NO:** Nitric oxide. Values are presented as mean ± **SD:** an Independent Sample-T test or b Mann Whitney-U test calculated p-values. Statistically significant p values are marked in bold.

comparisons between two groups were performed using the Mann-Whitney U test, and comparisons among three or more groups were perform using the Kruskal-Wallis test. A significance level of $p < 0.05$ was considered for all analyses. In order to determine the statistical power of the study, power analysis was performed with the G*Power 3.1 program, targeting 80% power and 5% significance level in hypothesis tests. As a result of these analyses, it was determined that 80 participants for each group would be sufficient to determine the expected effect size.

Results

Table 1 compares the demographic and laboratory characteristics of the control group with those of the PE group. No significant differences were found between the control and preeclampsia groups in terms of maternal age, gestational age, gravida, haemoglobin, platelet count, AST, ALT, and creatinine levels ($p>0.05$). The mean BH4 level was 81.4±10 pg/mL in the control group and 67.8±8.9 pg/mL in the PE group, with a p-value of 0.001, indicating a statistically significant difference. The mean NO level was 15.2±6.7 μmol/L in the control group and 29.4±21.4 μmol/L in the PE group, also showing a statistically significant difference with a p-value of 0.001. The mean biopterin level was 177.4±15.7 nmol/L in the control group and 186.4±12.7 nmol/L in the PE group, with a p-value of 0.002, indicating statistical significance. However, there were no statistically significant differences in folate levels (9.3±4.6 ng/mL in the control group vs. 9.4±4.5 ng/mL in the PE group, p=0.802) or vitamin B12 levels (181±69 pg/mL in the control group vs. 187±87 pg/mL in the PE group, p=0.811). Table 2 shows the comparison of demographic and laboratory findings between different groups based on the presence of PE and folic acid supplementation. The groups were classified as follows: Group 1 (no PE, no folic acid supplementation, P(-)/F(-)), Group 2 (no PE, with folic acid supplementation, P(-)/F(+)), Group 3 (with PE, no folic acid supplementation, P(+)/F(-)), and Group 4 (with PE, with folic acid supplementation, P(+)/F(+)). No significant differences were found among all groups in terms of maternal age, gestational age, gravida, haemoglobin, platelet count, AST, ALT, creatinine, and homocysteine levels ($p>0.05$). The mean BH4 levels were as follows: Group 1, 78.9±9.7 pg/mL; Group 2, 82.4±10.1 pg/mL; Group 3, 68.9±10.3 pg/mL; Group 4, 67.0±7.9 pg/mL. The p-value for the differences among these four groups

Table 2: Comparison of Demographic and Laboratory Findings of PE and Control Group Patients According to Folic Acid Use Status

Characteristic	P(-)/F(-) (n=23) Mean ± S.D.	P(-)/F(+) (n=57) Mean ± S.D.	P(+)/F(-) (n=30) Mean ± S.D.	P(+)/F(+) (n=50) Mean ± S.D.	p-value
Age (years)	28 ± 5	29 ± 5	29 ± 5	30 ± 5	0.455b
Gestational Age (weeks)	30 ± 6	29 ± 5	32 ± 4	31 ± 4	0.246b
Gravida	4 ± 2	4 ± 2	4 ± 2	4 ± 2	0.727b
BMI (kg/m²)	27 ± 2	26 ± 2	27 ± 1	27 ± 1	0.011b
SBP (mmHg)	121 ± 7	120 ± 7	150 ± 5	148 ± 5	0.001b
DBP (mmHg)	78 ± 4	78 ± 5	90 ± 4	91 ± 5	0.001b
Haemoglobin (g/dL)	11.7 ± 3	12.4 ± 1.1	11.6 ± 1.3	12.0 ± 1.2	0.019a
PLT (x10³/μL)	242 ± 70	229 ± 57	230 ± 60	225 ± 57	0.861b
AST (U/L)	16 ± 5	20 ± 8	19 ± 7	24 ± 17	0.130b
ALT (U/L)	11 ± 6	15 ± 8	14 ± 10	18 ± 17	0.163b
Creatinine (mg/dL)	0.56 ± 0.07	0.58 ± 0.06	0.62 ± 0.13	0.61 ± 0.12	0.244b
Homocysteine (μmol/L)	6.97 ± 4.4	7.59 ± 5.02	9.79 ± 6.44	7.75 ± 4.05	0.183b
Albumin (g/dL)	30 ± 3	31 ± 3	27 ± 4	28 ± 5	0.001b
Total Protein (mg/dL)	65 ± 4	65 ± 5	61 ± 7	62 ± 6	0.002b
Urinary Protein (mg/L)	139 ± 53	137 ± 46	1448 ± 2835	1882 ± 3957	0.001b
BH4 (pg/mL)	78.9 ± 9.7	82.4 ± 10.1	68.9 ± 10.3	67.0 ± 7.9	0.001a
Biopterin (nmol/L)	171.5 ± 14.9	179.3 ± 15.6	189.2 ± 8.1	184.9 ± 14.5	0.004b
NO (μmol/L)	15.9 ± 7.9	14.9 ± 6.2	28.4 ± 19.8	29.9 ± 22.5	0.001b
Folate (ng/mL)	5.8 ± 2.4	10.7 ± 4.6	6.7 ± 3.5	11.1 ± 4.3	0.001b
Vitamin B12 (pg/mL)	169 ± 64	187 ± 71	177 ± 95	193 ± 82	0.521b

BMI: Body mass index, **SBP:** Systolic blood pressure, **DBP:** Diastolic blood pressure, **PLT:** Platelet count, **AST:** Aspartate Aminotransferase, **ALT:** Alanine Aminotransferase, **BH4:** Tetrahydrobiopterin, **NO:** Nitric oxide. Values are presented as mean ± SD, **n:** number *p*-values were calculated with [a]One-way ANOVA or [b]Kruskal Wallis test. The significant pairwise group comparison results were as represented below;
For BH4: 1 to 3: p= 0.001, 1 to 4: p=0.001, 2 to 3: p=0.001, 2 to 4: p=0.001, (Post Hoc test: with Bonferroni correction). For Biopterin: 1 to 3: p=0.018, 2 to 3: p=0.30 (Post Hoc test: Tamhane's test). For NO: 1 to 3: p=0.018, 1 to 4: p=0.001, 2 to 3 p=0.006.

was 0.001, indicating a statistically significant difference (p=0.001). Formean BH4 values; there is a significant difference between group 1 and groups 3 and 4 and between group 2 and group 3 and 4. The values are higher in groups 1 and 2. The mean biopterin levels were: Group 1, 171.5±14.9 nmol/L; Group 2, 179.3±15.6 nmol/L; Group 3, 189.2±8.1 nmol/L; Group 4, 184.9±14.5 nmol/L. The p-value for these differences was 0.004, indicating statistical significance (p=0.004). There is a significant difference between Group 1 and Group 3 for the mean Biopterin value. The values are lower in group 1. The mean NO levels were: Group 1, 15.9±7.9 μmol/L; Group 2, 14.9±6.2 μmol/L; Group 3, 28.4±19.8 μmol/L; Group 4, 29.9±22.5 μmol/L. The p-value for these differences was =0.001, indicating a statistically significant difference (p=0.001). For mean NO values; there is a significant difference between group 1 and groups 3 and 4 and between group 2 and group 3. The values are lower in groups 1 and 2. The mean vitamin B12 levels were: Group 1, 169±64 pg/mL; Group 2, 187±71 pg/mL; Group 3, 177±95 pg/mL; Group 4, 193±82 pg/mL. The p-value for these differences was 0.521, showing no statistically significant difference (p>0.05)

Discussion

The study successfully demonstrates a significant association between serum tetrahydrobiopterin (BH4) levels, folic acid supplementation, and the development of preeclampsia (PE). BH4 levels were notably lower in the PE group, indicating its potential involvement in the pathogenesis of PE, particularly in endothelial dysfunction and nitric oxide (NO) synthesis. However, while folic acid supplementation improved serum folate and haemoglobin levels, no direct relationship between folic acid and the prevention of PE was established. These findings suggest that while folic acid plays a beneficial role in maternal health, its influence on PE risk is less clear. Overall, this study supports the hypothesis that BH4 may serve as a key marker in assessing preeclampsia risk, though additional clinical and biochemical parameters must be considered for a comprehensive diagnosis and treatment strategy.
The underlying mechanisms contributing to the pathophysiology of preeclampsia (PE) remain

incompletely understood. However, both genetic and environmental factors play significant roles in its development. As a multifactorial disorder, PE originates at the maternal-fetal interface and impacts multiple organs. The placenta is central to the progression of PE, and its removal typically resolves the clinical manifestations of the disease (10). Nitric oxide (NO), synthesized from L-arginine by nitric oxide synthase (NOS), is a key mediator in endothelium-dependent vasodilation. This molecule reduces peripheral vascular resistance during pregnancy and is essential for maintaining vascular function in various organs. Impaired endothelial function and increased oxidative stress have been implicated in the pathogenesis of PE and other conditions such as miscarriage and polycystic ovary syndrome (PCOS) (11,12,13). Although research on BH4 deficiency in PE is limited, Toth et al. hypothesized that adequate vitamin C levels in the placenta may protect the NO/eNOS pathway by stabilizing BH4. Decreased vitamin C levels in PE could contribute to BH4 depletion, leading to impaired NO production. Additionally, Kukor et al. reported variability in placental BH4 concentrations between PE and healthy pregnancies, noting that in some PE patients, eNOS becomes resistant to BH4 stimulation. This results in eNOS uncoupling, reduced NO production, and increased superoxide generation, suggesting oxidative stress as a key driver of vascular dysfunction in PE (14). Numerous studies have examined potential preventive and therapeutic strategies for PE. One area of focus has been folic acid metabolism and its relationship to homocysteine levels, which are elevated in hypertensive pregnancy disorders. Although folic acid supplementation has been shown to reduce homocysteine levels, its effect on PE risk remains unclear. Some studies indicate a potential benefit of folic acid supplementation in lowering PE risk, while others provide conflicting results (15). Moreover, insufficient folic acid intake has been associated with adverse pregnancy outcomes, including miscarriage, placental abruption, and fetal growth restriction (FGR) (16). Some researchers have explored whether folic acid supplementation can reduce the risk of FGR and preterm birth, but the results remain controversial. For example, some studies suggest that high maternal folate levels reduce the risk of preterm birth, while others link elevated folate levels early in pregnancy to an increased risk of gestational diabetes (17,18). The variability in outcomes highlights the need for further investigation into the role of folic acid in pregnancy. Emerging evidence suggests that the concentration of folic acid in fetal circulation may exceed that in maternal circulation, potentially leading to the accumulation of inactive metabolites in the fetus. Experimental studies indicate that even moderate increases in folic acid intake during pregnancy can alter placental gene expression related to angiogenesis, potentially influencing pregnancy outcomes (19). Several studies have demonstrated that folic acid supplementation, particularly in the first trimester, is associated with a reduced risk of severe PE (20). A Canadian study also reported a significant reduction in PE rates in women who took ≥1.0 mg of folic acid daily, particularly those at increased risk (21). These findings suggest that extending folic acid supplementation beyond the first trimester and using higher doses may help prevent PE by supporting physiological angiogenesis. Despite this, some studies have shown no significant relationship between folic acid supplementation and PE prevention (22). A systematic review concluded that the evidence for folic acid-reducing PE risk is weak (23), and a multicenter randomized controlled trial found no benefit from high-dose (4 mg/day) folic acid supplementation in preventing PE in twin pregnancies (24,25).

Study limitations: The most important limitation of this case-control study is the high risk of recall bias due to its retrospective nature. Participants' inability to accurately recall past exposures may affect the accuracy of the results. Additionally, accurate matching of cases and controls may be difficult and potential confounding variables may not be fully controlled. The generalizability of the findings is also limited because case-control studies may not reflect the general population.

Conclusion

This study revealed significantly lower serum BH4 levels in pregnant women with PE compared to controls. Additionally, folic acid supplementation was found to increase serum folate levels, with statistically significant differences observed between PE and control groups in terms of BH4, biopterin, NO, albumin, total protein, and urinary protein levels. These findings suggest that factors affecting vascular function, such as BH4 and NO, may play a critical role in PE pathogenesis. While no direct relationship between folic acid supplementation and PE development was established, folic acid use was shown to improve serum folate and haemoglobin levels, underscoring its importance

in general maternal health and pregnancy outcomes. However, BH4 and folic acid levels alone are insufficient to predict PE risk; instead, these factors should be considered alongside other clinical parameters for a more comprehensive assessment. Given the complexity of PE, further large-scale studies are needed to explore the role of BH4 and folic acid metabolism in its development. Future research should focus on understanding the precise mechanisms by which these molecules influence PE pathogenesis and evaluating their potential as therapeutic targets. This study provides valuable insights into the early diagnostic markers of PE and lays the groundwork for future research aimed at improving maternal and fetal outcomes.

Ethical approval: Written permission was obtained from the University's Non-Interventional Clinical Research Ethics Committee to conduct the research. Written consent was obtained from the patients stating that they participated in the study and provided the information used voluntarily.

Conflict of interest: The authors declare no conflict of interest fort his study.

Financial support: The author disclosed that they did not receive any grant during conduction or writing of this study.

Author contributions: Concept (MAA, AG, KU), Design (MAA, AG, KU), Data Collection and/or Processing (MAA, AG, KU), Analysis and/or Interpretation (MAA, AG, KU)

References

1. Chen CW, Jaffe IZ, Karumanchi SA. Pre-eclampsia and cardiovascular disease. Cardiovasc Res 2014;101(4):579-586
2. Rana S, Lemoine E, Granger JP, Karumanchi SA. Preeclampsia: Pathophysiology, Challenges, and Perspectives. Circ Res 2019;124(7):1094-1112.
3. Abbasi R, Bakhshimoghaddam F, Alizadeh M. Major dietary patterns in relation to preeclampsia among Iranian pregnant women: a case-control study. J Matern Fetal Neonatal Med 2021;34(21):3529- 3536.
4. Crider KS, Qi YP, Yeung LF, Mai CT, Head Zauche L, Wang A, et al. Folic Acid and the Prevention of Birth Defects: 30 Years of Opportunity and Controversies. Annu Rev Nutr 2022;42:423- 452.
5. Dodds L, Fell DB, Dooley KC, Armson BA, Allen AC, Nassar BA, et al. Effect of homocysteine concentration in early pregnancy on gestational hypertensive disorders and other pregnancy outcomes. Clin Chem 2008;54(2):326-334
6. Wadhwani NS, Patil VV, Mehendale SS, Wagh GN, Gupte SA, Joshi SR. Increased homocysteine levels exist in women with preeclampsia from early pregnancy. J Matern Fetal Neonatal Med 2016;29(16):2719-2725
7. Pánczél Z, Supák D, Kovács B, Kukor Z, Valent S. Effect of pravastatin on tetrahydrobiopterinsensitive and -resistant NO synthase activity of preeclamptic placentas. Orv Hetil 2020;161(10):389-395.
8. ACOG Committee on Obstetric Practice. "ACOG practice bulletin. Diagnosis and management of preeclampsia and eclampsia. Number 33, January 2002. American College of Obstetricians and Gynecologists." *International journal of gynaecology and obstetrics: the official organ of the International Federation of Gynaecology and Obstetrics* 77.1 (2002): 67-75.
9. Cochrane KM, Mayer C, Devlin AM, Elango R, Hutcheon JA, Karakochuk CD. Is natural (6s)-5-methyltetrahydrofolic acid as effective as synthetic folic acid in increasing serum and red blood cell folate concentrations during pregnancy? a proof-of-concept pilot study. Trials 2020; (21): 1-12.
10. Sircar M, Thadhani R, Karumanchi SA. Pathogenesis of preeclampsia. Curr Opin Nephrol Hypertens 2015;24(2):131–138.
11. Liu Y, Burdon KP, Langefeld CD, Beck SR, Wagenknecht LE, Rich SS et al. T-786C polymorphism of the endothelial nitric oxide synthase gene is associated with albuminuria in the diabetes heart study. J Am Soc Nephrol 2005;16(4):1085–1090.
12. K Turan, K Uckan, E Sarikaya, H Demir, C Demir. Investigation of prolidase, adenosine deaminase, glutathione s-transferase and glutathione reductase activities in patients with abortus imminens. j. clin. anal. med. 2017;8(6):519-522
13. K Uçkan, H Demir, Y Başkıran, C Demir. Investigation of activities enzyme prolidase (pro) and glutathione s-transferase (gst) in polycystic ovary syndrome (pcos) patients. J. Sci. Rep. .2022;050:20-31.
14. Tóth M, Kukor Z, Valent S. Chemical stabilization of tetrahydrobiopterin by L-ascorbic acid: contribution to placental endothelial nitric oxide synthase activity. Mol Hum Reprod 2002;8(3):271-280.

15. Liu C, Liu C, Wang Q, Zhang Z. Supplementation of folic acid in pregnancy and the risk of preeclampsia and gestational hypertension: a meta-analysis. Arch. Gynecol. Obstet. 2018;298:697-704.
16. Kaldygulova L, Ukybassova T, Aimagambetova G, Gaiday A, Tussupkaliyev A. Biological Role of Folic Acid in Pregnancy and Possible Therapeutic Application for the Prevention of Preeclampsia. *Biomedicines* 2023:*11:*272.
17. Ferrazzi E, Tiso G, Di Martino D. Folic acid versus 5- methyl tetrahydrofolate supplementation in pregnancy. Eur J Obstet Gynecol Reprod Biol 2020;253:312-319.
18. Saccone G, Berghella V. Folic acid supplementation in pregnancy to prevent preterm birth: a systematic review and meta-analysis of randomized controlled trials. Eur J Obstet Gynecol Reprod Biol 2016;199:76-81.
19. Luan Y, Leclerc D, Cosín-Tomás M, Malysheva OV, Wasek B, Bottiglieri T, et al. Moderate Folic Acid Supplementation in Pregnant Mice Results in Altered Methyl Metabolism and in Sex-Specific Placental Transcription Changes. Mol Nutr Food Res 2021;65(14):e2100197.
20. Wang Y, Zhao N, Qiu J, He X, Zhou M, Cui H, et al. Folic acid supplementation and dietary folate intake, and risk of preeclampsia. Eur J Clin Nutr 2015;69(10):1145-1150.
21. McNulty B, McNulty H, Marshall B, Ward M, Molloy AM, Scott JM, et al. Impact of continuing folic acid after the first trimester of pregnancy: findings of a randomized trial of Folic Acid Supplementation in the Second and Third Trimesters. Am J Clin Nutr 2013;98(1):92-98.
22. Ayala-Ramírez P, Serrano N, Barrera V, Bejarano JP, Silva JL, Martínez R, et al. Risk factors and fetal outcomes for preeclampsia in a Colombian cohort. Heliyon 2020;6(9):e05079.
23. Bulloch RE, Lovell AL, Jordan VMB, McCowan LME, Thompson JMD, Wall CR. Maternal folic acid supplementation for the prevention of preeclampsia: A systematic review and meta-analysis. Paediatr Perinat Epidemiol 2018;32(4):346-357.
24. Antoniades C, Shirodaria C, Warrick N, Cai S, de Bono J, Lee J, et al. 5-methyltetrahydrofolate rapidly improves endothelial function and decreases superoxide production in human vessels: effects on vascular tetrahydrobiopterin availability and endothelial nitric oxide synthase coupling. Circulation 2006;114(11):1193-1201.
25. Yuyun MF, Ng LL, Ng GA. Endothelial dysfunction, endothelial nitric oxide bioavailability, tetrahydrobiopterin, and 5-methyltetrahydrofolate in cardiovascular disease. Where are we with therapy? Microvasc Res 2018;119:7-12