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Correlation of mid-trimester homocysteine, vitamin B12 and folate levels with adverse pregnancy outcomes: A clinical study

İkinci trimester homosistein, B12 vitamini ve folat düzeylerinin olumsuz obstetrik sonuçlarla ilişkisi: Klinik çalışma

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Abstract

Aim: The aim of this study is to determine if maternal serum levels of folate, vitamin B12 and homocysteine at 20-26 weeks of gestation are associated with subsequent adverse pregnancy outcomes.

Materials and Methods: This prospective study was conducted in a tertiary care center and included 159 pregnant women at 20-26 gestational weeks. Complicated and uncomplicated pregnancy groups were compared in terms of serum levels of folate, vitamin B12, and homocysteine using Mann-Whitney U and t tests. Correlation of homocysteine with vitamin B12 and folate were analyzed with regression analysis.

Results: Of the 159 participants, 32 (20.13%) suffered from pregnancy complications. There were no significant differences in the levels of the midtrimester homocysteine (p=0.25) and vitamin B12 (p=0.19) between healthy and complicated pregnancy groups. The significant difference in terms of folate levels (p=0.02) was considered to be clinically insignificant, since the measured levels were within normal range. There is a statistically significant inverse correlation between homocysteine and vitamin B12 levels (r = -0.407, p=0.013).

Conclusion: Our results indicate that homocysteine, vitamin B12 and folate concentrations in mid-trimester are not associated with adverse pregnancy outcomes. These findings suggest that these markers seem not to have predictive value for pregnancy complications.

Keywords: Complication, folate, homocysteine, midtrimester, pregnancy, vitamin B12.

Öz

Amaç: *Bu çalışmanın amacı, gebeliğin 20-26. haftalarında bakılan maternal serum folat, B12 vitamini ve homosistein düzeylerinin gelişebilecek olumsuz obstetrik sonuçlarla ilişkisini araştırmaktır.*

Gereç ve Yöntem: *Bu klinik çalışma, üçüncü basamak bir merkezde gebeliğinin 20-26. haftasında olan 159 gebe üzerinde prospektif olarak yapıldı. Gebeliğin takibinde komplikasyon gelişen ve gelişmeyen gruplar, serum folat, B12 vitamini ve homosistein düzeyleri açısından t test ve Mann-Whitney U testi kullanılarak karşılaştırıldı. Homosisteinin, B12 vitamini ve folat ile korelasyonu için regresyon analizi kullanıldı.*

Bulgular: *Takipte 159 gebenin 32'sinde (%20.13) gebeliğe bağlı komplikasyon gelişti. Normal ve komplike gebelik grupları arasında, ikinci trimesterde bakılan homosistein (p=0.25) ve B12 vitamini (p=0.19) açısından anlamlı bir farklılık bulunmadı. Folat düzeyleri açısından istatistiksel olarak anlamlı farklılık tespit edildimesine (p=0.02) rağmen sonuçların normal sınır aralığında olmasından dolayı klinik olarak anlamsız olarak yorumlandı. Homosistein ve B12 vitamini seviyeleri arasında istatistiksel olarak anlamlı şekilde ters korelasyon (r = -0.407, p=0.013) tespit edildi.*
Sonuç: *Sonuçlarımız, ikinci trimesterde bakılan homosistein, B12 vitamini ve folat düzeylerinin gelişebilecek gebelik komplikasyonları ile ilişkisi olmadığını göstermektedir. Bu bulgulara göre homosistein, B12 vitamini ve folatın gebelik komplikasyonları konusunda prediktif bir değer taşımamaktadır.*

Anahtar Sözcükler: *Komplikasyon, folat, homosistein, ikinci trimester, gebelik, B12 vitamini.*

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Introduction

Homocysteine, a sulphur-containing amino acid, is derived from the demethylation of methionine during DNA and RNA methylation (1). Hyperhomocysteinemia prevalence in the general population is reported to be 5-7%, while this rate rises to 20-30% in atherosclerosis patients (2). Normal plasma levels of homocysteine vary between 5-15 μM/L. Among the causes of hyperhomocysteinemia are hereditary enzyme deficiencies, systemic diseases (chronic renal failure, acute lymphoblastic leukemia, hyperthyroidism, carcinomas of the breasts and ovaries, systemic lupus eryhtematosus), use of certain pharmaceuticals (e.g. methotrexate, nitric oxide, phenytoin) and dietary deficiencies (vitamins B6, B12, and folate) (3).

Growing evidence suggests that excessive homocysteinemia may exert pathological effects through oxidative damage and apoptosis. It is plausible that these metabolic events contribute to placental vascular dysfunction and to the maternal endothelial dysfunction that has been linked to adverse pregnancy outcomes (4).

Vascular-related pregnancy complications are a major cause of maternal and fetal morbidity and mortality. Maternal hyperhomocysteinemia is associated with placenta mediated diseases, such as preeclampsia, spontaneous abortion, intrauterine growth retardation and placental abruption. As diagnosis and treatment of hyperhomocysteinemia is relatively straightforward, monitoring and medical intervention of this condition is expected to either prevent and/or reduce the associated pregnancy complications (5,6).

Elevated homocysteine concentrations can be handled by folate and vitamin B12 supplementation (7). In addition, folate use may improve the endothelial function independent from its impact on homocysteine (8,9). From this point of view, folate and vitamin B12 have been of interest. The aim of the current study is to investigate the predictive potentials of the serum levels of homocysteine, folate and vitamin B12 for midtrimester pregnancy complications. In cases of hyperhomocysteinemia, early diagnosis, prophylactic intervention and appropriate care may prove to be valuable for achievement of a healthy pregnancy outcome.

Materials and Methods

A prospective cohort study was conducted at the obstetrics and gynecology department of our tertiary care center. A total of 159 pregnant women at 20-26 weeks of gestation, were included in the study and followed until delivery after obtaining informed consent. Performance of this study was approved by the local Institutional Review Board.

Blood samples were collected from the antecubital veins of the participants. Following venopuncture and collection of fasting samples, the blood was kept on ice until the separation of plasma, serum and cells within 60 minutes in the laboratory. Samples were centrifuged and serum was separated and stored at -80ºC until analysis.

The participants were allocated into two groups depending on the occurrence of complications or otherwise. Parameters of interest (homocysteine, folate and vitamin B12) were measured to determine if there were statistically significant differences, and whether these differences may have predictive values for pregnancy complications appearing after the second trimester.

Plasma levels of homocysteine were measured using the DS30 total homocysteine kit (*Drew Scientific Inc, Dallas, TX, 75237 USA*). Serum vitamin B12 and folate levels were determined by chemiluminescence using the BIO-DPC kit (*Roche Modular E170, Roche Diagnostics Turkey, Sisli, 34394 Istanbul, Turkey*).

Statistical Analysis

Data were analyzed using the Statistical Package for Social Sciences (SPSS) software (version 10.0 for Windows). All differences associated with a chance probability of .05 or less were considered statistically significant. Continuous variables are presented as mean±SD. T-test was used for the comparison of the values for homocysteine, while comparison of vitamin B12, and folate were performed via Mann-Whitney U Test. Correlation of homocysteine levels with vitamin B12, folate, gestational week, birth weight, age, and gravidas were investigated with regression analysis.

Results

Pregnant women were allocated into two groups with respect to the occurrence of complications during the monitoring process in mid-trimester. Of the 159 cases in total, 32 (20.13%) suffered from pregnancy complications. Four (%12.5) premature birth, 2 (%6.3) preeclampsia, 11 (%34.4) oligohydramnios, 1 (%3.1) ablatio placenta, 5 (%15.6) gestational diabetes mellitus, 3 (%9.4) intrauterine death, 4 (%12.5) preterm premature membran rupture, 2 (%6.3) intrauterin growth restriction were reported. Table 1 shows the comparison of normal and complicated pregnancies in terms of age, gravida, parity, number of children alive, and gestational weeks. The average age of complicated pregnants were higher than uncomplicated cases (p=0.01) and the gestational age at birth was significantly lower in complicated patients (p=0.01).

The values for homocysteine, vitamin B12, and folate in two groups are presented in Table-2. The folate levels were 12.7 (3.9-24.0) ng/ml, and 16 (6.9-24.0) ng/ml in

those with normal and complicated pregnancies, respectively, which constitutes a statistically significant difference ($p<0.05$). However, since folate levels remained within the normal range in both groups, this difference was considered to be clinically insignificant. There was no significant difference between the two groups for the other parameters measured. Data concerning the correlation of homocysteine levels with vitamin B12, folate, gestational weeks, birth weight, age, and gravidas in the study participants is presented in Table 3. There is a statistically significant inverse correlation between homocysteine and vitamin B12 levels ($r = -0.407$ $p<0.05$). The reverse is also correct in that the higher vitamin B12, the lower the homocysteine levels.

Table-1. Comparison of Normal and Complicated Pregnancies in Terms of Age, Gravida, Parity, Number of Living Children and Gestational Week.

	Normal pregnancy	Complicated pregnancy	p
Age	27.0 ± 5.2	29.8 ± 5.2	0.010*
Gravida	2.0 ± 1.2	2.1 ± 1.4	0.884
Parity	0.8 ± 0.9	0.6 ± 0.9	0.443
Number of living children	0.7 ±0.9	0.6 ± 0.9	0.594
Gestational weeks	39.1 ± 1.7	37.3 ± 3.7	0.010*

*: Statistically significant, $p<0.05$.

Table-2. Comparison of Complicated and Uncomplicated Pregnancies for Homocysteine, vitamin B12, Folic Acid and Blood Lipids.

	Normal pregnancy	Complicated pregnancy	p
Homocysteine (µM/L)	7.4 ± 2.6	6.8 ± 2.6	0.247
Vitamin B12 (ng/mL)	172 (129-402)	197 (136-331)	0.189
Folate (ng/mL)	16 (6.9-24.0)	12.7 (3.9-24.0)	0.023*

*: Statistically significant, $p<0.05$.

Table-3. Correlation of Homocysteine Levels with Vitamin B12, Folate, Gestational Week, Birth Weight, Age and Gravidas in our Series.

	Homocysteine (r)	p
Birth weight	-0.281	0.102
Folate	-0.009	0.960
Vitamin B12	-0.407	0.013*
Age	-0.079	0.642
Gravidas	-0.079	0.646

*: Statistically significant, $p<0.05$.

Discussion

Placental-related disorders of pregnancy affect around a third of human pregnancies (10). Oxidative stress in utero-placental tissues plays an important role in the development of placental-related diseases (11). Increased homocysteine levels represent a risk factor in cardiovascular disease, cerebrovascular disease, osteoporosis, renal failure, diabetic microangiopathy, neuropsychiatric disorders and adverse pregnancy outcomes (12,13).

Elevated plasma levels of homocysteine results in vascular endothelial damage. This undesirable effect occurs subsequent to the endothelial toxicity of the free radicals formed by the oxidation of homocysteine and the interference of homocysteine with the coagulation mechanism in a way that facilitates a prothrombotic state and clot formation (14).

Numerous studies investigated the homocysteine and its association with pregnancy and pregnancy-linked complications (15-18). These studies have confirmed that mild hyperhomocysteinemia is associated with vascular-related pregnancy complications, such as pre-eclampsia, recurrent miscarriages and intrauterine growth retardation (19-21). Wouters et al. (22) were the first to report a correlation between repeated hyperhomocysteinemia and intrauterine growth retardation (IUGR). In this study, 14% of the patients with primary repeated IUGR had hyperhomocysteinemia. The essence of the hypothesis is that hyper-homocysteinemia in early pregnancy causes damage to the decidual and chorionic vasculature. De Vries and colleagues determined that there was hyperhomocysteinemia in 38% of women with intra-uterine growth retardation and in 11% of those with intrauterine fetal deaths unrelated to pre-eclampsia (23). Leeda et al. (24) performed methionine loading tests on women with intrauterine growth retardation post-partum and found that 19.2% had hyperhomocysteinemia. It has been demonstrated that homocysteine levels are higher in preeclamptic women than those in normal pregnants (25-27).

However, vast majority of the publications in the literature, homocysteine levels in complicated pregnancies were evaluated at the end of pregnancy period or after the complication is clinically obvious. Our study is original in terms of design since we measured plasma homocysteine levels during 20-26 weeks of gestation prior to the existence of complications. We came across no significant differences between normal and complicated cases with regard to levels of homocysteine, vitamin B12 and folate. Therefore, they seemed to have no predictive value for the likelihood of complication development. Similar to our study, Sorensen and colleagues came across no significant difference in the levels of plasma homocysteine in 52 patients with pre-eclampsia and 56 normo-tensive pregnants on the 16^{th} week of gestation (28).

There is a strong correlation between increasing concentrations of homocysteine and decreasing amounts of folate in the plasma. In agreement with previous studies, the folate levels measured during the second trimester were lower in women who developed complications than those who did not. The average folate levels in complicated and uncomplicated pregnancies were 12.7 (3.9-24.0) ng/mL and 16 (6.9-24.0) ng/mL, respectively (p=0.023). Even though this difference is statistically significant, it is clinically insignificant because the values still remain within the normal range for both groups. Levels of vitamin B12 showed no significant difference in both groups.

Homocysteinemia treatment varies depending on the cause. The intended levels of homocysteine are accomplished by increasing re-methylation or trans-sulphuration. The cofactors for the enzymes that participate in homocysteine metabolism, cystathionine ß-synthase and methyltetrahydrofolate reductase, are vitamin B6, vitamin B12 and folate respectively. Daily supplementation of vitamin B6 (100-250 mg/day), vitamin B12 (1-2 mg/day) and folate (5-10 mg/day) is recommended for acceleration of homocysteine metabolism. In response to multivitamin treatment, levels of homocysteine decline within 2-6 weeks in over 90% of the patients. Dietary modifications may also affect homocysteine levels in the blood (29-30).

Several maternal lifestyle factors, such as smoking, alcohol consumption and folic acid supplement use, and weight as proxy of nutrition and lifestyle, affect the homocysteine, folate and vitamin B12 status. Our results, although observational and only indicative of causal relations, emphasize the importance of optimizing these pre-conceptional nutrition and lifestyle behaviours.

Limitations of our study are our relatively small series and lack of definite criteria for selection of patients for this method. In addition, some details of history and factors that may influence the outcome may not be completely documented. Due to these restrictions, associations should be interpreted with caution. However, we hope that this study will pioneer not only further studies on this topic and aid in the development of alternative options for diagnosis and management of pregnancy-related complications.

Homocysteinemia is believed to constitute a risk factor for pre-eclampsia and other gestational complications. Since hyperhomocysteinemia is a well-known and treatable condition, the importance of the determination of homocysteine levels becomes apparent in those with a history of obstetric problems such as pre-eclamsia in their previous pregnancies. When homocysteinemia is detected, treatment with folate, vitamins B6 and B12 can be considered for the management of later pregnancies

Conclusion

Our results indicate that levels of folate, vitamin B12 and homocysteine in maternal plasma did not differ significantly at 20-26th weeks of gestation in complicated or uncomplicated cases. Therefore, these substances seem not to have a predictive value for adverse materno-fetal outcomes in the second trimester. Further prospective, randomized, double-blinded clinical trials in larger series may help to unveil their actual diagnostic potential.

References

1. Leach NV, Dronca E, Vesa SC, et al. Serum homocysteine levels, oxidative stress and cardiovascular risk in non-alcoholic steatohepatitis. Eur J Intern Med 2014;25(8):762-7.
2. Sun Q, Jia X, Gao J, et al. Association of serum homocysteine levels with the severity and calcification of coronary atherosclerotic plaques detected by coronary CT angiography. Int Angiol 2014;33(4):316-23.
3. Perna AF, Castaldo P, Ingrosso D, De Santo NG. Homocysteine, a new cardiovascular risk factor, is also a powerful uremic toxin. J Nephrol 1999;12(4):230-40.
4. Hague WM. Homocysteine and pregnancy. Best practice & research. Clin Obstet Gynaecol 2003;17(3):459-69.
5. Ray JG, Laskin CA. Folic acid and homocyst(e)ine metabolic defects and the risk of placental abruption, pre-eclampsia and spontaneous pregnancy loss: A systematic review. Placenta 1999;20(7):519-529.
6. Furness D, Fenech M, Dekker G, Khong TY, Roberts C, Hague W. Folate, vitamin B12, vitamin B6 and homocysteine: Impact on pregnancy outcome. Matern Child Nutr 2013;9(2):155-66.
7. Homocysteine Lowering Trialists Collaboration. Dose-dependent effects of folic acid on blood concentrations of homocysteine: A meta-analysis of the randomized trials. Am J Clin Nutr 2005;82(4):806-12.
8. Doshi SN, McDowell IF, Moat SJ, Lang D, Newcombe RG, Kredan MB. Folate improves endothelial function in coronary artery disease: an effect mediated by reduction of intracellular superoxide? Arterioscler Thromb Vasc Biol 2001; 21(7):1196-202.
9. Doshi SN, McDowell IF, Moat SJ, Payne N, Durrant HJ, Lewis MJ. Folic acid improves endothelial function in coronary artery disease via mechanisms largely independent of homocysteine lowering. Circulation 2002;105(1):22-6.
10. Jauniaux E, Poston L, Burton GJ. Placental-related diseases of pregnancy: Involvement of oxidative stress and implications in human evolution. Hum Reprod Update 2006;12(6):747-55.
11. Acilmis YG, Dikensoy E, Kutlar AI, et al. Homocysteine, folic acid and vitamin B12 levels in maternal and umbilical cord plasma and homocysteine levels in placenta in pregnant women with pre-eclampsia. J Obstet Gynaecol Res 2011;37(1):45-50.
12. Ueland PM, Nygard O, Vollset SE, Refsum H. The Hordaland Homocysteine Studies. Lipids 2001;36 Suppl:S33-39.

13. Refsum H, Smith AD, Ueland PM, Nexo E, Clarke R, McPartlin J. Facts and recommendations about total homocysteine determinations: An expert opinion. Clinical Chemistry 2004;50(1):3-32.
14. Micle O, Muresan M, Antal L, Bodog F, Bodog A. The influence of homocysteine and oxidative stress on pregnancy outcome. J Med Life 2012;5(1):68-73.
15. Cotter AM, Molloy AM, Scott JM, Daly SF. Elevated plasma homocysteine in early pregnancy: A risk factor for development of severe preeclampsia. Am J Obstet Gynecol 2001;185(4):781-85.
16. Kim MW, Hong SC, Choi JS, et al. Homocysteine, folate and pregnancy outcomes. J Obstet Gynaecol 2012;32(6):520-4.
17. Powers RW, Evans RW, Majors AK, Ojimba JI, Ness RB, Crombleholme WR. Plasma homocysteine concentration is increased in preeclampsia and is associated with evidence of endothelial activation. Am J Obstet Gynecol 1998;179(6):1605-11.
18. Guerra-Shinohara EM, Paiva AA, Rondo PH, Yamasaki K, Terzi CA, D'Almeida V. Relationship between total homocysteine and folate levels in pregnant women and their newborn babies according to maternal serum levels of vitamin B12. BJOG 2002;109(7):784-91.
19. Vollset SE, Refsum H, Irgens LM, Emblem BM, Tverdal A, Gjessing HK. Plasma total homocysteine, pregnancy complications, and adverse pregnancy outcomes: The Hordaland Homocysteine study. Am J Clin Nutr 2000;71(4):962-8.
20. Steegers-Theunissen RP, Steegers EA. Nutrient-gene interactions in early pregnancy: a vascular hypothesis. Eur J Obstet Gynecol Reprod Biol 2003;106(2):115-7.
21. Bergen NE, Jaddoe VW, Timmermans S, Hofman A, Lindemans J, Russcher H. Homocysteine and folate concentrations in early pregnancy and the risk of adverse pregnancy outcomes: The Generation R Study. BJOG 2012;119(6):739-51.
22. Wouters MG, Boers GH, Blom HJ. Hyperhomocysteinemia: A risk factor in women with unexpected recurrent erly pregnancy loss. Fertil Steril 1993;60(5):820-5.
23. De Vries JI, Dekker GA, Huijgens PC, Jakobs C, Blomberg BM, Van Geijn HP. Hyperhomocysteinaemia and protein S deficiency in comlicated pregnancies. Br J Obstet Gynaecol 1997;104(11):1248-54.
24. Leeda M, Riyazi N, de Vries JI, Jakobs C, van Geijn HP, Dekker GA. Effects of folic acid and vitamin B6 supplementation on women with hyperhomocysteinemia and a history of preeclampsia or fetal growth restriction. Am J Obstet Gynecol 1998;179(1):135-9.
25. Mujawar SA, Patil VW, Daver RG. Study of serum homocysteine, folic acid and vitamin b(12) in patients with preeclampsia. Indian J Clin Biochem 2011;26(3):257-60.
26. Khosrowbeygi A, Ahmadvand H. Circulating levels of homocysteine in preeclamptic women. Bangladesh Med Res Counc Bull 2011;37(3):106-9.
27. Khosrowbeygi A, Ahmadvand H. Positive correlation between serum levels of adiponectin and homocysteine in pre-eclampsia. J Obstet Gynaecol Res 2013;39(3):641-6.
28. Sorensen TK, Malinow MR, Williams MA, King IB, Luthy DA. Elevated second trimester serum homocysteine levels and subsequent risk of preeclampsia. Gynecol Obstet Invest 1999;48(2):98-103.
29. Hankey GJ, Ford AH, Yi Q, et al. VITATOPS Trial Study Group. Effect of B vitamins and lowering homocysteine on cognitive impairment in patients with previous stroke or transient ischemic attack: A prespecified secondary analysis of a randomized, placebo-controlled trial and meta-analysis. Stroke 2013;44(8):2232-9.
30. Ciaccio M, Bellia C. Hyperhomocysteinemia and cardiovascular risk: Effect of vitamin supplementation in risk reduction. Curr Clin Pharmacol 2010;5(1):30-6.