Investigation of Folic Acid, Vitamin B12, Vitamin B6 and Homocysteine Levels in Preeclamptic Pregnancies

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Abstract

Objective: To investigate the relationship between preeclampsia and serum folic acid, serum B12, plasma vitamin B6 (pyridoxal-5'-phosphate) and plasma homocysteine levels.

Methods: 100 pregnant women with preeclampsia and 74 healthy pregnant women were included in the study from September 2004 to August 2005. Serum vitamin B12, folic acid and plasma vitamin B6 and homocysteine levels were measured in all patients at their third trimester of pregnancy.

Results: Vitamin B12 serum levels in the preeclamptic and the control group were similar (117.44±42.03 pg/ml in the preeclamptic group and 136.07±59.01 pg/ml in the control group, p>0.05). Vitamin B6 (pyridoxal-5'-phosphate) plasma levels in preeclampsia group were lower than control group (6.40±2.91µg/l in preeclampsia group and 11.15±5.76 µg/l in the control group p<0.001). Folic acid serum levels in the preeclampsia group were lower (5.43±3.08 ng/ml in the control, 8.00±5.1 ng/ml, in the preeclamptic group, p<0.001). Homocysteine plasma levels were significantly higher in the preeclampsia group than control group (10.50±6.21 µmol/L in the preeclamptic group and 6.54.±2.64 µmol/L in the control group, p<0.001).

Conclusion: High homocysteine level and low folic acid and vitamin B6 levels play a role in the pathogenesis of preeclampsia.

Keywords: Preeclampsia, homocysteine, folate, vitamin B6, vitamin B12.

Preeklamptik gebelerde folik asit, vitamin B12, vitamin B6 ve homosistein düzeylerinin araştırılması

Amaç: Preeklampsi ile serum folik asit, serum vitamin B12, plazma vitamin B6 (piridoksal 5 fosfat) ve plazma homosistein düzeyleri arasındaki ilişkinin araştırılması.

Yöntem: Eylül 2004-Ağustos 2005 tarihleri arasında, kliniğimize başvuran ve preeklampsi tanısı alan 100 gebe ile herhangi bir medikal problemi olmayan 74 sağlıklı gebe çalışmaya dahil edildi. Bütün hastalardan gebeliğin 3. trimesterinde serum vitamin B12, serum folik asit, plazma vitamin B6 ve plazma homosistein konsantrasyonları ölçüldü.

Bulgular: Vitamin B12 serum düzeyleri preeklampsi ve kontrol grupları arasında benzerdi (sırası ile; 117.44±42.03 pg/ml, 136.07±59.01 pg/ml, p>0.05). Vitamin B6 (piridoksal 5 fosfat) plazma düzeyi preeklampsi grubunda kontrol grubuna göre düşük bulundu (sırası ile; 6.40±2.91 µg/l, 11.15±5.76 µg/l, p<0.001). Folik asit serum düzeyi preeklampsi grubunda daha düşük saptandı (sırası ile; 5.43±3.08 ng/ml, 8.00±5.1 ng/ml, p<0.001). Homosistein plazma düzeyi ise preeklampsi grubunda kontrol grubuna göre anlamlı oranda yüksekti (sırası ile; 10.50±6.21 µmol/L, 6.54±2.64 µmol/L, p<0.001).

Sonuç: Yüksek homosistein konsantrasyonu ile düşük folik asit ve vitamin B6 düzeyleri preeklampsinin patogenezinde rol oynayabilir.

Anahtar Sözcükler: Preeklampsi, homosistein, folik asit, vitamin B6 (piridoksal 5 fosfat), vitamin B12.

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Introduction

Homocysteine is an aminoacid that forms during methionine metabolism. Homocysteine blood levels are affected by genetic or many acquired factors. The lack of enzymes added to the homocysteine metabolism or the cofactors necessary for its metabolism (folate, B6 vitamin, B12 vitamin) cause hyperhomocysteinemia.[1] Mc Cully was the first to suggest that high homocysteine leads to atherosclerotic and thrombotic complications.[2] Further studies showed disorder in endothelium dependant relaxation in various isolated artery preparations incubated with homocysteine.[3] The placental vascular changes in preeclamptic patients: due to similar changes to those of atherosclerotic patients, various studies have been done to investigate the relationship between preeclampsia and homocysteine, vitamin B6 (pyridoxal-5'-phospate), vitamin B12 and folic acid levels, and it was shown in these studies that preeclamptic patients have higher homocysteine levels than healthy pregnant women, and that there are changes in parallel to this in vitamin B6 (pyridoxal-5'-phosphate), vitamin B12 and folic acid levels.[4,5]

The objective of this study is to research whether there is any difference between preeclamptic pregnant women and healthy normotensive pregnant women through the comparison of serum folic acid, serum vitamin B12, plasma vitamin B6 (pyridoxal-5'-phosphate) and plasma homocysteine levels.

Methods

This study was performed prospectively on a total of 174 normotensive healthy pregnant women and pregnant women diagnosed with preeclampsia who do not use any medication other than the iron preparation and do not smoke and who contacted the Clinics of Gynecology and Obstetrics, Faculty of Medicine, Dicle University between September 2004 and August 2005. Ethical approval was received to perform the study. Furthermore, approval was also received from all patients that their participation in the study is voluntary. Preeclamptic pregnant women were classified as the study group and healthy pregnant women as the control group. The preeclampsia classification was done according to ACOG (American College of Obstetricians and Gynecologists) criteria.[6] Blood pressure ≥ 140/90 mmHg and proteinuria > 300 mg/dl in the 24 hour urine sample was considered to be mild preeclampsia; blood pressure ≥ 160/110 mmHg and proteinuria (5 gr/more than 24 hours), oliguria (500 ml/less than 24 hours), headache, sight impairment, pain in the right upper quadrant, liver function failure, and thrombocytopenia were considered severe preeclampsia criteria.

In addition to the obstetric evaluation and laboratory examination in the 3rd trimester of gestation, blood was taken from all patients into EDTA tubes for homocysteine and vitamin B6 (pyridoxal-5'-phosphate) analysis, and into plain gel tubes for folic acid and vitamin B12 analysis. After all samples were centrifuged at 3500 rpm for 10 minutes, plasma was allocated for homocysteine and vitamin B6 (pyridoxal-5'-phosphate) and serum for folic acid and vitamin B12. The samples acquired were stored in the dark and at -24ºC until the day of the study.

Homocysteine levels were studied using a DPC brand "Immulate 2000 Homocysteine" kit (reference range 5.0-12 µmol/L), on the Immulate 2000 apparatus (DPC, Los Angeles) using the immunoassay method.

Vitamin B6 (pyridoxal-5'phosphate) levels were studied using a Recipe brand "ClinRep Complete Kit for vitamin B6 (pyridoxal-5'phosphate) in Plasma and Whole Blood" kit (reference range 8.6-27.2 µg/l), on the HPLC apparatus (Schimadzu, Japan) using the chromatographic method.

Folic acid levels were studied using a Roche brand kit (reference range 3.1-17.5 µg/l), on the E170 system (Roche, Los Angeles) using the chemiluminescence immunoassay method.

Vitamin B12 levels were studied using a Roche brand kit (reference range 30-2000 pg/ml), on the E170 system (Roche, Los Angeles) using the chemiluminescence immunoassay method. The student t test SPSS 10.0 for Windows computer program was used in the statistical analysis of data, and $p<0.05$ was accepted statistically significant.

Results

A total of 174 cases were included in the study of which 100 were preeclampsia cases (Group 1) and 74 were healthy pregnancy cases (Group 2).

Table 1. The demographic, hematological and biochemical parameters of the groups and the weights of the newborn.

Parameters	Preeclampsia (Average and SD)	Healthy pregnancy (Average and SD)	P
Age	30.28±6.40	29.70±5.59	p>0.05
Gravida	5.00±3.54	4.90±3.40	p>0.05
Parity	3.37±3.34	3.02±3.13	p>0.05
Systolic (mm/Hg)	158.30±16.57	113.78±11.66	*p<0.001
Diastolic (mm/Hg)	96.45±11.74	71.48±8.55	*p<0.001
Gestational week	34.15±4.09	38.06±1.29	*p<0.001
White blood cell (/ mL)	13.812±5.0	11.577±3.3	*p<0.001
Hemoglobin (g/dL)	11.3±2.0	11.6±1.5	p>0.05
Hematocrit (%)	38.68±5.91	34.56±4.08	p>0.05
Trombosis (K/uL)	215.2±103	270±62	*p<0.001
LDH (U/L)	509.2±395.7	202.2±88.9	*p<0.001
AST (U/L)	94.39±20.30	20.06±7.74	*p<0.001
ALT(U/L)	67.73±17.82	17.62±7.28	*p<0.001
Albumin (g/L)	2.76±0.60	3.28±0.36	*p<0.001
Proteinuria (mg/dL)	365.3±189.1	14.0±6.1	*p<0.001
Apgar 1	4.65±2.65	6.01±1.94	*p<0.001
Apgar 5	6.42±3.05	8.13±1.46	*p<0.001
Weight at birth (g)	2228.50±88.86	3245.54±398.68	*p<0.001

*Statistically significant.

Cases were separated into two groups: preeclamptic and healthy pregnant women. In group 1 cases (n=100), mild preeclampsia was predicted in 74 cases (74%) and severe preeclampsia in 26 cases (26%). The demographic, hematological and biochemical parameters, and newborn weights and first and 5th minute Apgar scores of the cases are shown in Table 1. No statistically significant difference was predicted between the age, gravid, parities between both groups (p>0.05). The week of delivery, birth weight, first and 5th minute apgar scores between both groups were statistically significant (p<0.001).

No statistical difference could be predicted between the preeclampsia and control groups in terms of vitamin B12 serum level (respectively, 117.44±42.03 pg/ml, 136.07±59.01 pg/ml, p>0.05) (Diagram 1).

The vitamin B6 (pyridoxal-5'-phosphate) plasma level was found to be low in the preeclampsia group in comparison to the control group (respectively, 6.40±2.91 µg/l, 11.15±5.76 µg/l p<0.001) (Diagram 2).

Folic acid serum level was found to be lower in the preeclampsia group (respectively, 5.43±3.08 ng/ml, 8.00±5.1 ng/ml, p<0.001) (Diagram 3).

And the homocysteine plasma level was found to be at a significantly high ratio in the preeclampsia group in comparison to the healthy pregnancy group (respectively, 10.50±6.21 µmol/L, 6.54±2.64 µmol/L, p<0.001) (Diagram 4) (Table 2).

Furthermore, no statistical difference could be predicted in terms of the vitamin B12, vitamin B6, folic acid and homocysteine serum levels of mild and severe preeclamptic patients (p>0.05).

Discussion

Homocysteine is methionine's non-essential amino acid derivative. High plasma homocysteine levels are a risk factor for endothelial dysfunction, vascular disease, aterosclerosis.[1-5]

Endothelial damage plays a role in the etiology of preeclampsia. It leads to vascular endothelial damage at high homocysteine levels. Therefore, the relationship between high homocysteine levels and preeclampsia has been shown in various studies.[6] De Vries et al have predicted that 24% of preeclamptic patients also have high homocysteine.[8] It is considered that high homocysteine levels may also make vascular endothelia in preeclamptic patients more sensitive towards oxidasive stress.[9] In their study, Kassab et al have predicted that high homocysteine leads to maternal hypotension, proteinuria, renal damage, intrauterine developmental retardation, and increased fetal mortality.[10] Patrick et al have shown that decreased folic acid and B12 levels cause high homocysteine concentrations in preeclamptic patients.[11] In their

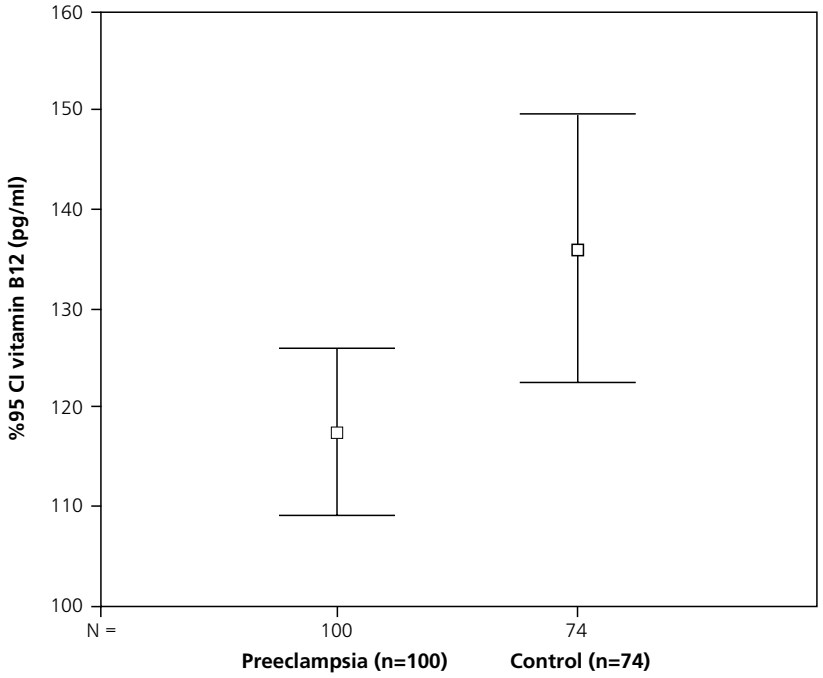

Diagram 1. Vitamin B12 levels of the group of preeclampsia and healthy pregnant women.

study, Gürbüz et al have shown that there is a prevalent degree of high homocysteine concentration in preeclamptic patients, and that high homocysteine concentrations are in parallel with the bad prognosis of preeclampsia.[12] Calle et al have stated that vitamin B6 (pyridoxal-5'-phosphate) and vita-

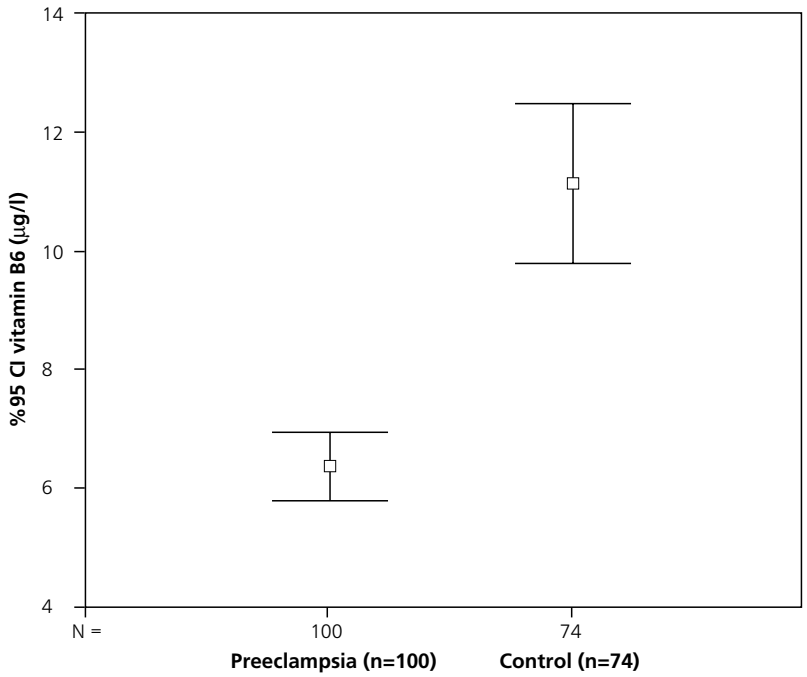

Diagram 2. Vitamin B6 levels of the group of preeclampsia and healthy pregnant women.

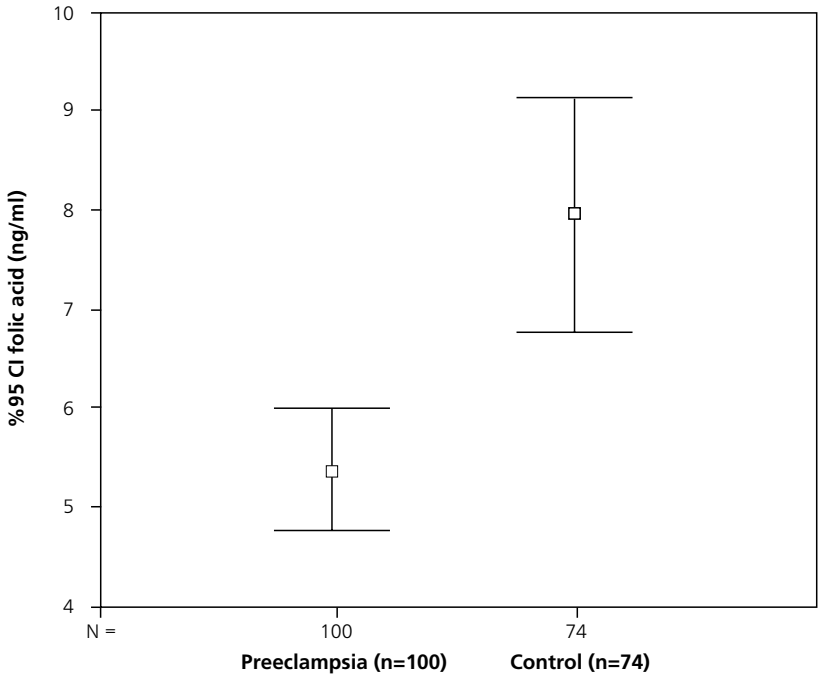

Diagram 3. Folic acid levels of the group of preeclampsia and healthy pregnant women.

min B12 play a role in the homocysteine metabolism, and that the administration of these vitamins during the term of gestation with a diet prevent complications such as spontaneous low intrauterine developmental retardation, preeclampsia, intrauterine morbidity linked to gestation.[13] In their

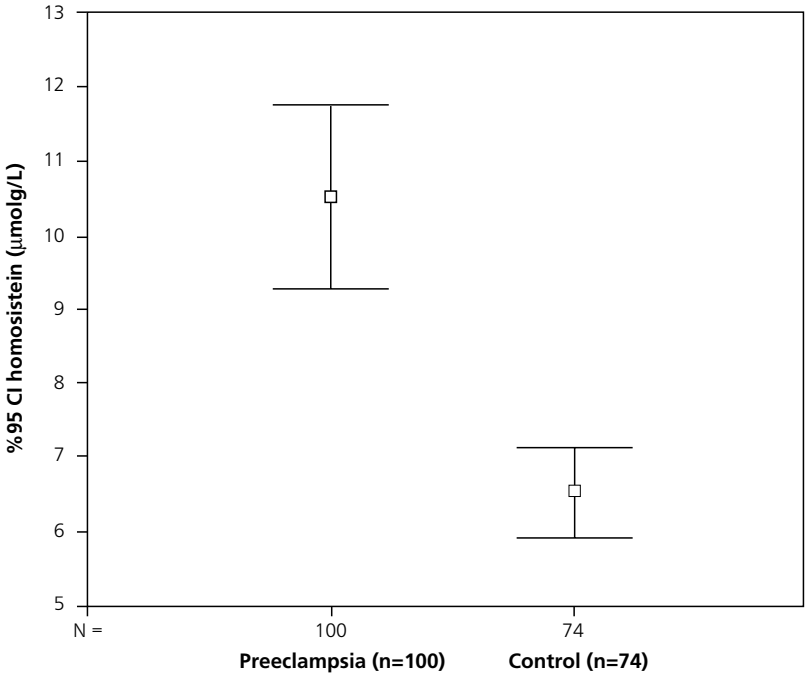

Diagram 4. Homocysteine levels of the group of preeclampsia and healthy pregnant women.

study, while predicting homocysteine levels as higher in the preeclampsia pregnancy group in comparison to the healthy pregnancy group, Üstüner et al have predicted that serum folic acid levels are low and have stated that it is necessary to correct the lack of nutritional folic acid during the antenatal term.[14] The lack of folic acid in pregnant women receiving folic acid treatment in the second and third trimester of gestation was predicted to be less in comparison to those not receiving the treatment. Thence, with the consideration that 30% of preeclamptic gestations will repeat, it is suggested that folic acid treatment be started to observe the homocysteine level in preeclamptic pregnant women and to prevent its repetition in subsequent pregnancies.[15,16]

According to data from this study, plasma homocysteine concentrations being predicted as higher and folic acid and vitamin B6 levels as lower in preeclamptic pregnant women in comparison to healthy pregnant women gives rise to the thought that high homocysteine concentration and low folic acid and vitamin B6 levels may play a role in the pathogenesis of preeclampsia. If preeclampsia and high homocysteine level are predicted together during the gestational term, we believe that it is necessary to inform the patient in terms of possible risks and to closely monitor the gestation.

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