

A comparative study of folate and vitamin B₁₂ serum levels in preeclamptic versus normotensive pregnant women in correlation with uterine and umbilical artery Doppler findings and pregnancy outcome

Normotansif ve preeklamptik gebelerde serum folat ve vitamin B12 düzeylerinin uterin ve Umbilikal arter Doppler sonuçları ve gebelik sonuçlarıyla karşılaştırılması

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Abstract

Objective: To detect the serum levels of folate and B12 in both preeclamptic and normotensive pregnant women and to determine whether there is any relation between these levels with the uterine and umbilical artery Doppler indices as well as the pregnancy outcome.

Material and Methods: This case controlled study comprised 79 pregnant patients with preeclampsia and 113 healthy, normotensive pregnant women with singleton pregnancies at gestational ages ranging from 34- 40 weeks. Patients were not obese (BMI<30) and did not suffer from chronic hypertension, chronic renal or liver disease nor diabetes mellitus. Serum folate and B12 were detected in all cases. They were also subjected to a Doppler study of both the uterine and umbilical arteries. Serum folate and B12 blood levels as well as the Doppler study indices (RI and PI) were compared in both groups.

Results: The serum folate level was significantly lower in preeclamptic patients than normal pregnant women (p<0.001). It was significantly correlated to uterine artery Doppler indices (RI and PI) and negatively correlated to umbilical artery Doppler indices (RI and PI). Low serum folate was significantly correlated to poor maternal outcome. Low serum folate was also significantly correlated to poor perinatal outcome. Serum B12 level was not significantly different in preeclamptic patients from the control group (P value=0.14).

Conclusion: Serum folate was significantly lower in preeclamptic pregnant women with a significant correlation to increased uterine and umbilical RI, PI and poor maternal and neonatal outcome.

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Key words: Preeclampsia, serum folate, serum vitamin B12, umbilical artery Doppler, uterine artery Doppler

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Özet

Amaç: Normotansif ve preeklamptik gebelerde serum folat ve vitamin B12 düzeylerini uterin ve umbilikal arter doppler sonuçları ve gebelik sonuçlarıyla karşılaştırmak.

Gereç ve Yöntemler: Bu vaka kontrol çalışması 34-40 haftalara arasındaki 79 preeklamptik ve 113 normotansif gebe ile yapıldı. Hastalar obez değillerdi (BMI<30) ve kronik hipertansiyon, böbrek veya karaciğer rahatsızlığı olanlar ile, diabetli hastalar çalışmaya alınmadı. Serum folat ve vitamin B12 düzeyleri tüm vakalarda bakıldı. Ayrıca uterin ve umbilikal arter Doppler çalışması yapıldı.

Bulgular: Preeklamptik hastalarda serum folat düzeyleri anlamlı olarak daha düşük idi (p<0.001). Bu uterin arter Doppler indeksleri ile pozitif, umbilikal arter Doppler indeksleri ile negatif yönde korele idi. Düşük serum folat seviyeleri kötü obstetrik sonuç ile ilişkili idi. Serum B12 vitamin seviyeleri farklı değildi.

Sonuç: Serum folat seviyeleri preeklamptik gebelerde anlamlı olarak daha düşüktür ve uterin arter Doppler indeksleri ve kötü obstetrik sonuç ile koreledir.

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Anahtar kelimeler: Preeklampsi, serum folat, serum vitamin B12, umbilikal arter Doppleri, uterin arter Doppleri

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Introduction

Pre-eclampsia is a disease with worldwide significance. In developing countries, it accounts for 20–80% of the maternal

mortality. Folate and vitamin B₁₂ play an important role in the metabolism of homocysteine. Many studies prove hyperhomocysteinemia to be a factor that causes endothelial injury in pre-eclampsia. Perinatal mortality of infants of pre-eclamptic

mothers is five-fold greater than for non pre-eclamptic women, and indicated preterm deliveries for pre-eclampsia account for 15% of preterm births (1).

Current concepts of the genesis of pre-eclampsia that include endothelial dysfunction, inflammatory activation, oxidative stress and predisposing maternal factors provide targets for well-designed nutritional investigation (2).

The amount of homocysteine in the blood is regulated by three vitamins: folic acid, vitamin B₁₂, and vitamin B₆. Reduced folate intake or genetic abnormalities of folate metabolism are associated with increased serum homocysteine concentration (3). Many studies prove hyperhomocysteinemia, to be a factor that causes endothelial injury in pre-eclampsia (4-6).

Recent interest has emerged addressing folate deficiency and various pregnancy complications e.g. pre-eclampsia (7,8), abruptio placentae (9), neural tube defects (10), preterm deliveries (11), intra-uterine growth restriction (12) in addition to other non pregnancy related complications e.g. cardiovascular diseases (13), cognitive impairment (14) and cancer (15). Some investigators show decreased serum B₁₂ in pre-eclamptic patients while others demonstrate no change (5, 16-20).

Material and Methods

Study design: case-control study

Participants: This study was conducted at Kasr El-Aini hospital, Cairo, Egypt from March 2006 until February 2008. Two groups of patients were included in the study, 79 pregnant patients who were diagnosed as having pre-eclampsia and a control group included 113 normotensive pregnant women who were both age and gestational age matched. Preclampsia was defined by having a blood pressure above 140/90 measured at least twice, 6 hours apart and proteinuria >300mg/24hours or $\geq +1$ proteinuria in the absence of another cause of proteinuria suggested by urine analysis (1). All patient ages ranged from 20-35 years. They were all non obese (BMI < 30) and had a singleton pregnancy at 34-40 weeks gestation. The preclamptic patients included in the study did not suffer from chronic hypertension, chronic renal or liver disease or diabetes mellitus.

A full history was taken including age, gestational age, parity, symptoms of severity (in pre-eclamptic cases), state of vitamin supplementation (considered positive in case of regular intake of B complex containing vitamins throughout pregnancy), and blood pressure measurement.

Laboratory investigations

Routine investigations: blood samples were collected on EDTA tubes for CBC analysis as well as on plain tubes for alanine aminotransferase (ALT), and aspartate aminotransferase (AST) and creatinine which were analyzed using commercial kits on the Synchron CX5 analyzer (Beckman Instruments Inc., USA) (21). Fresh morning urine samples were also collected for complete urine analysis.

Special investigations: Serum folate and B₁₂: Venous samples were withdrawn from all patients after an overnight fast, centrifuged and serum was stored at -20°C until the time of analysis. Serum folate and B₁₂ were measured by a radioimmunoassay

kit (simulTRAC B12/folate -SNB 57Co/125I radioimmunoassay kit) (MP Biomedicals, USA). The procedure of analysis is as described by the manufacturer pamphlet. The sensitivity of the kit for folate is 1.38nmol/l, and for B₁₂ is 75pg/ml (Gutcho and Mansbach, 1977).

Ultrasound examination

Ultrasound assessment of uterine artery Doppler velocimetry by transabdominal technique with investigation of the main uterine artery at its crossing with the iliac vessels using the Accuvix (Medison, Korea) was made. Doppler indices were taken (RI and PI). The velocimetries of both uterine arteries were taken and the average indices were calculated. Assessment of umbilical artery velocimetry: using the same machine, a random site for the umbilical artery was determined and the Doppler indices were taken (PI, RI). Neonatal APGAR score, birth weight as well as admission to neonatal ICU were recorded.

Statistical analysis:

Data were statistically described in terms of mean \pm standard deviation (\pm SD), frequencies (number of cases) and relative frequencies (percentages) when appropriate. Comparison of quantitative variables between the study groups was done using Student t test for independent samples in comparing 2 groups when normally distributed, and Mann Whitney U test for independent samples when not normally distributed. For comparing categorical data, Chi square (χ^2) test was performed. Correlations between various variables were done using Pearson moment correlation equation. A probability value (p value) less than 0.05 was considered statistically significant. All statistical calculations were done using computer programs *Microsoft Excel version 7* (Microsoft Corporation, NY, USA) and *SPSS* (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) statistical programs for Microsoft Windows.

Results

This study showed that serum folate was significantly lower in pre-eclamptic patients versus normal pregnant women (9.4 \pm 8.8 nmol/L versus 20.2 \pm 13.9 nmol/L p < 0.001). On the other hand serum B₁₂ level was not significantly different in pre-eclamptic patients from the control group (350.7 \pm 283 pg/ml versus 424 \pm 364 pg/ml, p-value = 0.14) (Table 1). Folate supplementation was significantly lower in pre-eclamptic patients (5.1% in pre-eclamptic versus 34.5% in control group, p < 0.001).

Table 1. Difference in serum folate and B12 in pre-eclamptic versus control pregnant women

Group		Folate (nmol/L)	B ₁₂ (pg/ml)
PE cases (n=79)	Mean	9.4	350.7
	SD	8.8	283.1
Controls (n=113)	Mean	20.2	424.8
	SD	13.9	364.7
P-value		<0.001	0.14

The serum folate is significantly higher in supplemented group (21.5±13.8 nmol/L in supplemented versus 8.7±8 nmol/L in non supplemented group, p=0.037).

Uterine and umbilical artery Doppler velocimetry showed a statistical difference between pre-eclamptic patients and the control pregnant women: Uterine RI and PI were significantly higher in pre-eclamptic group (RI: 0.58±0.1 versus 0.44±0.07, p<0.001; PI: 1.05 ± 0.29 versus 0.67 ± 0.18, p<0.001). Umbilical RI, PI were significantly higher in pre-eclamptic patients (RI: 0.63±0.09 versus 0.56±0.07, p=0.008; PI: 1.027±0.27 versus 0.82±0.16, p<0.001).

Serum folate was significantly correlated to uterine artery RI (Pearson correlation=-0.43, p<0.001) and PI (Pearson correlation=-0.46, p<0.001) (Figure 1) as well as umbilical artery RI (Pearson correlation=-0.22, p=0.002), PI (Pearson correlation=-0.22, p=0.002) (Figure 2).

Our study demonstrates no significant difference in hemoglobin levels between pre-eclamptic and control groups, but a significant difference exists in systolic blood pressure (SBP), diastolic blood pressure (DBP), platelet count, ALT, AST, creatinine level, neonatal birth weight (NBW) (Table 2), neonatal APGAR score and neonatal ICU admission .

Our results showed that 12 newborns of the preeclampsia group (15.1 %) had an APGAR score at 5minutes of less than7 whereas 67 newborns of the preeclampsia group (84.4%) had an APGAR score at 5minutes equal to or more than 7. All the newborns of the control group 113(100%) had an APGAR score at 5minutes equal to or higher than 7.

59 newborns of preclamptic group (74.7%) had a history of admission to the neonatal intensive care unit, whereas the 20 other newborns (25.3%) had not been admitted there, also none of the newborns of the control group 113(100%) had not been admitted to the neonatal intensive care unit.

Low serum folate was significantly correlated to poor maternal outcome (higher maternal serum creatinine, Pearson correlation=-0.354 P<0.001, higher serum ALT (Pearson correlation=-0.213, p=0.004), higher serum AST (Pearson correlation=-0.244, p=0.001) and lower platelet count (Pearson correlation=-0.235, p=0.047) and poor perinatal outcome (preterm labour, low birth weight, low APGAR score-less than 7 at 5minutes- and neonatal ICU admission); the serum level in poor outcome cases was 8.53±9.37 nmol/L versus 18.9±13.4 nmol/L in good perinatal outcome cases, p<0.001 (Figure 3).

Discussion

Hypertensive disorders complicating pregnancy are common and form one of the deadly triad, along with hemorrhage and infection, which contribute greatly to maternal morbidity and mortality. How pregnancy incites or aggravates hypertension remains unsolved despite decades of intensive research. Indeed, hypertensive disorders remain among the most significant and intriguing unsolved problems in obstetrics.

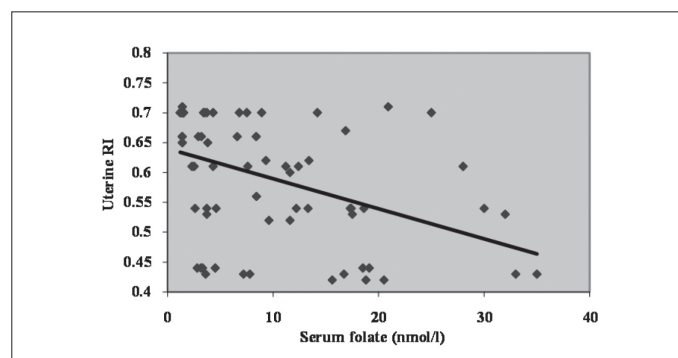


Figure 1. Correlation between serum folate (nmol/l) and uterine artery RI in Preeclamptic cases

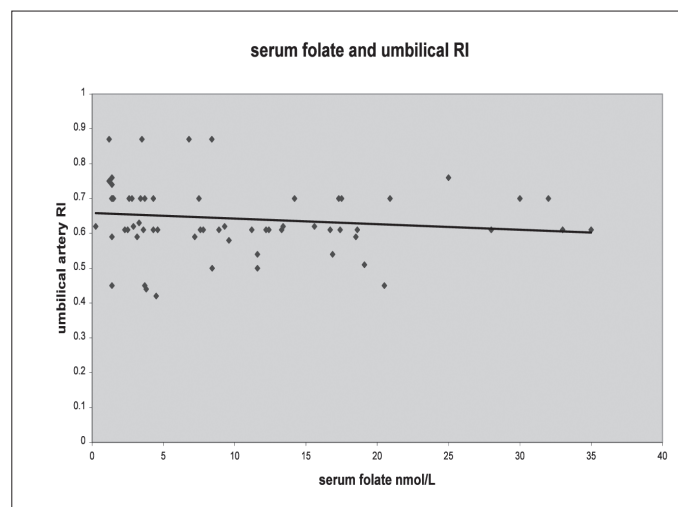


Figure 2. Correlation between serum folate (nmol/l) and umbilical artery RI in PE cases

Table 2. Mean and standard deviation of serum creatinine, ALT, AST, platelets and neonatal birth weight (NBW) in pre-eclamptic versus control pregnant women

Group		PLT (x103/μL)	ALT (U/L)	AST (U/L)	Creatinine (mg/dl)	NBW (g)
PE cases (n=79)	Mean	145.9	33.2	35.6	0.77	2359
	SD	55.2	44.8	44.2	0.16	4273
Controls (n=113)	Mean	192.9	11.4	11.9	0.48	3216
	SD	41.9	4.23	5.36	0.12	5035
P value		0.009	0.000	0.000	0.028	0.000
PLT: platelets						

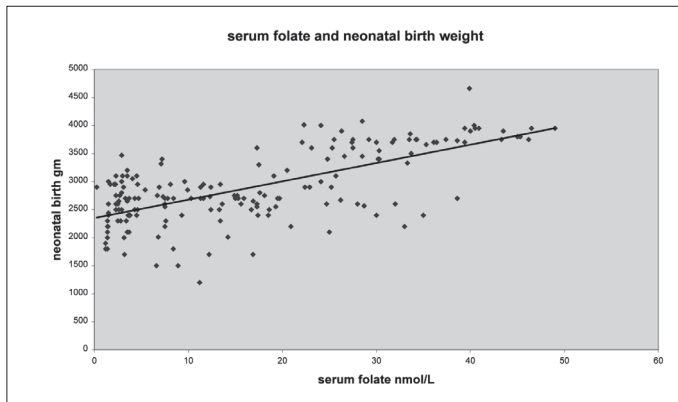


Figure 3. correlation between serum folate and neonatal birth weight

The neonatal birth weight in our study was significantly lower in the pre-eclamptic patients. This was similar to other previous studies (22-25). Neonatal intensive care unit admissions were significantly higher in pre-eclamptic patients. This result was also obtained by Ray, 2001(24).

Uterine RI and PI were significantly higher in the pre-eclamptic group. These results are supported by previous studies (26-28) and were explained by lack of normal trophoblastic invasion in pre-eclamptic patients. We found in our results that the umbilical RI, PI were significantly higher in pre-eclamptic patients. These results were in agreement with other studies (29-31).

Serum folate was significantly lower in pre-eclamptic patients versus normal pregnant women. This was the same evaluation as that reached by previous studies (7, 8, 32). The scientific plausibility of this result may be related to the important role of folate in the metabolism of amino acid homocysteine which has a protective role in the prevention of hyperhomocysteinemia, the same result as that by Holmes et al., 2005 (33). Hyperhomocysteinemia in turn is regarded a risk factor of pre-eclampsia (5, 34, 35) leading to endothelial injury and to hyper-coagulable states, as stated by El-Khairi, 2003(36). Folate is also important in DNA synthesis, so it may have an important role in trophoblastic proliferation and invasion of maternal spiral arteries, the derangement of which is a key feature in pre-eclampsia. This result of folate level disagreed with other previous studies (4, 6, 19). However, most of these studies were carried out on a small sample size and no adequate exclusion criteria were considered in the pre-eclamptic group. The view that there is a need for further research with adequate sample size was mentioned in a meta-analysis done by Ray et al. 2001(24). The possible protective role of folate in the prevention of pre-eclampsia is supported by our results regarding folate supplementation, which is significantly lower in pre-eclamptic patients (5.1% in pre-eclamptic versus 34.5% in control group, $p < 0.001$). The serum folate is significantly higher in the supplemented group (21.5 ± 13.8 nmol/L in supplemented versus 8.7 ± 8 nmol/L non supplemented group, $p = 0.037$). This is similar to the result of a study done by Hermann et al. 2005 study (8).

As regards the correlation of serum folate to uterine and umbilical artery Doppler velocimetry in our study, serum folate was significantly correlated to uterine artery RI and PI. This result

supported the hypothesis that folate may be involved in the pathogenesis of pre-eclampsia. No similar study reported this relation. Serum folate was also significantly correlated to umbilical artery RI and PI. This also supported the hypothesis that folate may be important in adequate trophoblastic proliferation including the tertiary stem villi. Also no similar study reported this relation to our knowledge.

In our study, there was a significant correlation between serum folate and both maternal and neonatal outcome: Low serum folate was significantly correlated to poor perinatal outcome parameters which involve preterm labour, low birth weight, low APGAR score –less than 7 at 5minutes- and neonatal ICU admission. This was similar to results presented by Mitchell et al. 2004(37). Low serum folate was significantly correlated to poor maternal outcome. As far as we know no similar studies showed these relations.

Our results showed that serum B_{12} level did not show any significant difference between both pre-eclamptic patients and the control group, and this was similar to other previous studies (7, 8) but was contradictory to the results reached by Laivouri et al. 1999(16) who found decreased serum B_{12} in pre-eclamptic patients, but this study's sample was small compared to ours (20 pre-eclamptic patients and 20 pregnant control). Their results may be explained by the fact that, in spite of the role of B_{12} in the metabolism of homocysteine, folate may be more important in this metabolism in protecting against pre-eclampsia.

The strength of our study was based on adequate sample size (79 pre-eclamptic patients and 113 controls), exclusion of known risk factors of pre-eclampsia to allow proper assessment and measuring serum folate and B_{12} much more accurately by blood sampling in the fasting state. However, there were some limitations in our study which deserve consideration. The serum level of folate in this cross-sectional study was significantly lower in pre-eclamptic patients versus control pregnant women, which proved the presence of association between low serum folate and the occurrence of pre-eclampsia. This association might be either a cause of pre-eclampsia or a result of some metabolic derangement in the course of the disease. It supported the idea that this association was most probably a cause, and was the role of folate in decreasing the homocysteine level and that vitamin supplementation was less likely to be present in pre-eclamptic patients. Some studies such as VanPampus et al. 1999. (38) demonstrated the presence of hyperhomocysteinemia long after delivery, supporting the view that hyperhomocysteinemia may be a cause of pre-eclampsia rather than an effect.

Since our pregnant women took either multivitamins containing folate (the majority) or B-complex vitamins throughout pregnancy, other confounding variables (the presence of other protective elements against pre-eclampsia in the multivitamin-minerals such as vitamin C, E) may affect our conclusion regarding the protective effect of folate against pre-eclampsia. But supported our conclusion that a significant correlation existed between low serum folate and poor maternal and neonatal outcome in pre-eclamptic patients and abnormal uterine and umbilical artery Doppler velocimetry findings. Also Hernandez-Diaz et al. 2002(39) demonstrated in their study that there

was a lower risk of pre-eclampsia in pregnancies with folate supplementation. However, due to the relatively small number of these studies, this area will need further research work.

In Conclusion, Serum folate was significantly lower in pregnancies complicated by pre-eclampsia. It was significantly correlated to abnormal uterine and umbilical artery Doppler velocimetry and poor maternal and neonatal outcome, So folate deficiency might have a role in the aetiology of pre-eclampsia and also folate supplementation throughout pregnancy might decrease the incidence of pre-eclampsia. Folate supplementation throughout pregnancy, and folic acid fortification of food could help to decrease the risk of pre-eclampsia especially in poor socioeconomic pregnant women who had an inadequate folate intake. There was no significant difference in serum B₁₂ level in pre-eclamptic versus control pregnant women but further studies would be needed to support our results.

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