The evaluation of homocysteine level in patients with preeclampsia

Ocena poziomu homocysteiny u pacjentek ze stanem przedrzucawkowym

Fatih Şanlıkan[1], Fatma Tufan[2], Ahmet Göçmen[1] ,Ceyda Kabadayı[3], Erkan Şengül[1]

[1] Ümraniye Education and Research Hospital, Department of Obstetrics and Gynecology İstanbul, Turkey
[2] Mahmudiye Family Health Center, Eskişehir, Turkey
[3] Bahçelievler Nazife Yaman Family Health Center, İstanbul, Turkey

Abstract

Objectives: *The aim of the study was to evaluate the correlation between preeclampsia and blood plasma homocysteine levels.*

Material and methods: *The research was conducted in a group of 114 pregnant patients who were subdivided into three groups consisting of: 30 women with severe preeclampsia, 24 with mild preeclampsia, and 60 healthy pregnant controls. Patient data included age, parity, body mass index (BMI), systolic and diastolic blood pressure, homocysteine, folic acid, vitamin B12, hematocrit, hemoglobin, blood urine nitrogen, uric acid and urine analysis.*

Results: *There were no differences in the demographic characteristics (age, gravidity and BMI) among the groups. Mean serum homocysteine level was significantly higher in the preeclamptic group as compared to controls ($p<0.01$). Mean homocysteine level in the control group was significantly lower than in the severe and mild preeclampsia groups, respectively ($p<0.001$ vs. $p<0.05$). There were no statistically significant differences in homocysteine levels between mild and severe preeclampsia groups ($p>0.05$). Although there were statistically significant differences among the three groups in terms of BUN, creatinine, AST, ALT, and LDH, no statistically significant differences in serum folic acid, vitamin B12 and hemoglobin levels were found.*

Conclusions: *Plasma homocysteine levels are significantly elevated in patients with preeclampsia and are not correlated with disease severity.*

Key words: **homocysteine** / **preeclampsia** / **folic acid** /

Corresponding author:
Fatih Şanlıkan
Adem Yavuz Cad. No 1 Kadın Hastalıkları ve Doğum Kliniği, Ümraniye, Turkey
Tel.: +90 216 632 18 18; fax: +90 (0216) 632 71 24
e-mail: fatihroland@hotmail.com

Otrzymano: **03.06.2014**
Zaakceptowano do druku: **12.12.2014**

Streszczenie

Cel: *Celem badania była ocena związku pomiędzy stanem przedrzucawkowym a poziomem homocysteiny w osoczu krwi.*

Materiał i metoda: *Badanie przeprowadzono w grupie 114 ciężarnych, które podzielono na trzy podgrupy: 30 kobiet z ciężkim stanem przedrzucawkowym, 24 z lekkim stanem przedrzucawkowym i 60 zdrowych ciężarnych. Analizowano następujące dane pacjentek: wiek, rodność, indeks masy ciała (BMI), ciśnienie skurczowe i rozkurczowe, homocysteinę, kwas foliowy, witaminę B12, hematokryt, hemoglobinę, mocznik, kwas moczowy i badanie ogólne moczu.*

Wyniki: *Nie znaleziono różnic w cechach demograficznych (tj. wiek, ilość ciąż i BMI) pomiędzy grupami. Średni poziom homocysteiny był istotnie wyższy u pacjentek ze stanem przedrzucawkowym niż w grupie kontrolnej ($p<0.01$). Średni poziom homocysteiny w grupie kontrolnej był istotnie niższy niż w grupie z ciężkim i lekkim stanem przedrzucawkowym, odpowiednio ($p<0.001$ vs. $p<0.05$). Nie stwierdzono istotnych różnic w poziomie homocysteiny pomiędzy łagodnym a ciężkim stanem przedrzucawkowym ($p>0.05$). Chociaż nie znaleziono istotnych różnic pomiędzy trzema badanymi grupami w odniesieniu do BUN, kreatyniny, AST, ALT, i LDH, to stwierdzono różnice (nieistotne statystycznie) w surowiczym poziomie kwasu foliowego, witaminy B12 i hemoglobiny.*

Wnioski: *Poziom homocysteiny w osoczu jest istotnie podwyższony u pacjentek ze stanem przedrzucawkowym ale nie koreluje z jego ciężkością.*

Słowa kluczowe: **homocysteina** / **stan przedrzucawkowy** /

Introduction

Preeclampsia is a hypertensive disorder of pregnancy, associated with widespread vascular endothelial malfunction and clinically defined by hypertension and proteinuria occurring after 20 weeks of gestation. According to the new ACOG (American College of Obstetricians and Gynecologists) diagnostic criteria for preeclampsia, the dependence of the diagnosis on proteinuria has been eliminated. In the absence of proteinuria, new-onset hypertension in association with thrombocytopenia, increased liver enzymes, renal insufficiency, pulmonary edema, cerebral or visual symptoms is also defined as preeclampsia [1]. Preeclampsia affects approximately 5% of all pregnant women [2, 3]. Despite developments in the field of obstetrics, preeclampsia constitutes one of the leading causes of maternal and fetal mortality and morbidity worldwide due to the fact that its exact etiopathogenesis remains unclear. The defined risk factors for preeclampsia include primiparity, previous preeclamptic pregnancy, chronic hypertension or chronic renal diseases or both, history of thrombophilia, multi-fetal pregnancy, *in vitro* fertilization, family history of preeclampsia, diabetes mellitus, obesity, systemic lupus eritromatosus, and advanced maternal age. There are many theories about the etiology of preeclampsia, including abnormal trophoblastic invasion of the uterine blood vessels, discordance of the immunologic tolerance between placental and fetal tissues, vascular endothelial cell damage, as well as dietary and genetic factors.

Homocysteine, a sulfur containing amino acid, is formed from methionine, an essential amino acid found in many animal and plant foods. Increased plasma homocysteine level is an independent risk factor for atherosclerosis and vascular thrombosis [4]. It has been shown that alterations in the methionine-homocysteine metabolism may be related to systemic vascular damage that leads to the classical clinical manifestation of preeclampsia. Oxidative stress and endothelial dysfunction caused by hyperhomosisteinemia may lead to preeclampsia. The aim of our study was to investigate the correlation between preeclampsia and plasma homocysteine levels.

Material and methods

A total of 114 pregnant patients who presented to the Ümraniye Education and Research Hospital, Obstetrics and Gynecology Department, were recruited for the study. Informed consents were obtained from the patients and Local Ethics Committee approved of the study. The research was conducted on 114 pregnant patients who were classified into three groups: 30 with severe preeclampsia, 24 with mild preeclampsia, and 60 healthy pregnant women. Mild and severe preeclamptic patients were classified as the research group, whereas healthy women constituted the control group. Patient data included age, parity, BMI, systolic and diastolic blood pressure, homocysteine, folic acid, vitamin B_{12}, hematocrit, hemoglobin, blood urine nitrogen, uric acid and urine analysis. The presence of imminent symptoms such as headache, epigastric tenderness, and visual symptoms were evaluated in the severe preeclamptic group.

The diagnosis of preeclampsia and severe preeclampsia was assessed according to the criteria of the National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy. The blood pressure (BP) of all participants was measured at rest. Patients with chronic hypertension, gestational diabetes, type 1 and type 2 diabetes, connective tissue disorders, chronic renal disease, multiple pregnancy, infectious diseases, intermarriage, alcohol users, and smokers were excluded from the study.

In order to assess the plasma homocysteine levels, venous blood samples were taken after 8 hours of fasting. Samples were collected as 2cc volume in EDTA containing tubes. Blood samples were centrifuged for 10 min. at 2500 rpm and plasma samples were kept in -20ºC until use. The homocysteine levels were analyzed with Immulite 2000 Immunoassay System (Siemens**,** Erlangen, Germany).

All calculations were carried out using NCSS (Number Cruncher Statistical System) 2007 and PASS 2008 Statistical Software (Utah, USA). Normally distributed continuous variables were reported as mean±standard deviation, non-normally distributed continuous variables were also reported as

mean±standard deviation and median, while discrete variables were reported as numbers and percentages of the total. Only gravity was reported as median and Interquartile Range (IQR). Mann-Whitney U test was used for the comparison of difference in medians between the preeclampsia and the control groups. One-way ANOVA was used for the comparison of normal distributions among the three groups. Kruskal Wallis was used for the comparison of non-normal distributions among the three groups. If differences occurred between the groups, Mann-Whitney U test with Bonferroni correction was used. The χ^2 and the Fisher-Exact tests were used for the comparison of proportions, if appropriate. Correlations between blood plasma homocysteine level and the other parameters for each group were expressed using Spearman Rank correlation analysis. Probability (p) values of less than 0.05 were considered as statistically significant.

Results

Mean patient age was 27.07±5.92 years. Patients were classified into three groups: severe preeclampsia (n=30), mild preeclampsia (n=24), and the control group (n=60). Blood samples were obtained from 114 pregnant women: 54 preeclamptic patients and 60 controls. Plasma homocysteine levels, biochemical parameters, whole blood count and urine analysis of all pregnant women were investigated. No differences among the groups in terms of the demographic characteristics (age, gravidity and BMI) were found (Table 1). Mean serum level of homocysteine was significantly higher for the preeclamptic group as compared to controls ($p<0.01$). Mean homocysteine level in the control group was significantly lower than in the severe and mild preeclampsia groups, respectively ($p<0.01$ vs. $p<0.05$). No statistically significant differences between homocysteine levels in the mild and severe preeclampsia groups were found ($p>0.05$). Although there were statistically significant differences between the three groups with regard to BUN, creatinine, ALT, AST, and LDH, there were no significant differences in serum folic acid, vitamin B12 and hemoglobin levels between the three groups (Table 1).

No statistically significant correlation between plasma homocysteine level and age, SBP, DBP, MBP for the preeclampsia and the control groups was found (Table II).

Discussion

Homocysteine is a naturally occurring amino acid, produced as a part of the body's methylation process. The level of homocysteine in the plasma is increasingly being recognized as a risk factor for vascular damage and seen as a potential predictor of health problems such as cardiovascular disease, neurological complications, cognitive impairment, congenital defects, and pregnancy complications [5]. Increased plasma levels of methionine, a metabolite of homocysteine, have been considered to cause oxidative stress and vascular endothelial dysfunction related to preeclampsia. It also affects nitric oxide synthesis and function. Elevated homocysteine is associated with damage to the arteries by interfering with the way cells use oxygen, resulting in a build-up of damaging free radicals [6].

Blood homocysteine concentration during pregnancy may be different. Lopez et al., found that homocysteine was significantly lower in all three trimesters of pregnancy as compared to non-pregnant controls [7]. In normal pregnancy, serum level of homocysteine decreases in the first trimester, reaching the minimum level in the second trimester and slightly increasing toward pre-pregnancy values in the second half of the third trimester [8]. There are numerous studies explaining the reasons for decreased homocysteine level during pregnancy. Refsum et al., reported that the cause of low homocysteine level during pregnancy was related to decrease of plasma albumin concentration [9]. Other reported causes included the decrease of vitamin B_{12} and folic acid plasma levels [10], physiological hemodilution in pregnancy [11], increased liver and renal enzyme activation due to high levels of estrogen and cortisol [12-13], or fetal usage of maternal homocysteine [14]. Malinow et al., showed that venous cord blood concentration of homocysteine was higher than arterial blood due to fetal consumption of maternal homocysteine [14].

Lopez et al., found an association between hyperhomosisteinemia and preeclampsia. In their study, the concentration of plasma homocysteine levels in patients with preeclampsia was higher than the control group and the odds ratio for preeclampsia in hyperhomosisteinemia patients was 7.7 (CI 95%, 1.7-34.8). Interestingly, they found that the level of folic acid in the preeclamptic group was higher than in controls [15]. Makedos et al., compared homocysteine, folic acid and vitamin B_{12} levels between 28 preeclamptic women and 26 healthy pregnant controls matched for gestational age [16]. They reported that the level of homocysteine was higher in women with preeclampsia than in the control group ($p < 0.001$). There were no differences between the groups regarding the levels of folic acid (11.12 vs. 9.73 ng/ml, $p=0.55$) and vitamin B_{12} (295.76 vs. 356.15 pg/ml, $p= 0.43$). In another study, Power et al., found high levels of homocysteine in preeclamptic patients as compared to the control group. In the same study, the level of fibronectin, a marker of endothelial dysfunction, was high in preeclamptic patients and the authors concluded that endothelial dysfunction was caused by hyperhomosisteinemia [17]. Var et al., investigated the relation between homocysteine and endothelial dysfunction. They found that NO level was low and homocysteine level was high in the preeclamptic group as compared to controls [18]. Similarly, Rajkoviç et al., confirmed a relationship between high levels of homocysteine and preeclampsia, but were unable to find any differences in folic acid and vitamin B_{12} concentrations between the groups [19]. In these studies, regardless of the severity of preeclampsia, maternal plasma levels of homocysteine were higher in preeclamptic patients than healthy controls. Dodds et al., in their prospective cohort study on the outcomes of 2119 patients, assessed the effect of homocysteine concentration in the early pregnancy and found that subjects with increased homocysteine concentrations were at increased risk of preeclampsia (RR 2.7, 95% CI 1.4-5.0) than subjects with lower homocysteine concentrations [20]. Their findings are consistent with our results, as in our study homocysteine levels were higher in the preeclamptic group than in the control group ($p<0.05$). There was no statistically significant difference between the homocysteine levels in the mild and severe preeclampsia groups (10.58±5.63 vs. 8.4±4.20; $p>0.05$). We found no differences in folic acid and vitamin B_{12} levels among the three groups, either ($p>0.05$).

On the other hand, there are numerous studies in the literature reporting no relationship between homocysteine level

Table I. The demographical properties and the measured parameters of the patients.

	Severe Preeclampsia	Mild Preeclampsia	Control	*p*
	Mean±SD	Mean±SD	Mean±SD	
Age	28,50±6,08	28,33±7,53	25,85±4,88	***0,067***
BMI	32,24±4,15	30,83±4,51	31,45±1,94	***0,284***
Gravidity (median (IQR))	2 (2)	1(1)	2(1)	***0,111***
Homocysteine	10,58±5,63 (9,87)[&]	8,94±4,20 (8,30)	6,61±2,34 (6,23)	***0,001***
Folic acid	8,99±3,70 (7,07)	8,66±2,79 (7,50)	9,15±4,04 (8,25)	***0,688***
Vitamin B_{12}	160,11±83,66 (123,50)	168,74±46,80 (180,00)	182,64±77,38 (178,35)	***0,191***
Hematocrit	35,80±5,49 (36,20)	33,04±3,96 (31,90)	36,87±2,75 (37,50)	***0,001***
Hemoglobin	11,72±1,91 (11,65)	11,04±1,45 (10,70)	11,71±1,13 (12,00)	***0,164***
BUN	18,90±6,46 (16)	16,54±4,93 (15)	12,53±3,53 (12)	***0,001***
Creatine	0,68±012 (0,67)	0,64±0,18 (0,57)	0,49±0,13 (0,40)	***0,001***
ALT	12,98±6,22 (11,5)	12,05±10,89 (8,4)	8,70±4,18 (7,5)	***0,001***
AST	20,69±8,76 (19,0)	17,11±8,41 (16,3)	13,07±3,95 (12,0)	***0,001***
LDH	278,48±95,10 (250)	286,71±92,46 (275)	147,50±24,12 (150)	***0,001***
Uric acid	5,23±1,46 (4,50)	4,73±1,09 (4,35)	4,03±0,74 (4,00)	***0,001***

[&] indicates median value

Table II. The correlation of homocysteine and Age, SBP, DBP and MBP or Preeclampsia and Control Group.

	Preeclampsia		Control	
	Homocysteine			
	r	*p*	*r*	*p*
Age				
Systolic blood pressure (SBP)	0,184	*0,183*	*0,011*	*0,931*
Diastolic blood pressure (DBP)	-0,188	*0,174*	*-0,067*	*0,610*
Mean Arterial Blood Pressure (MBP)	-0,040	*0,774*	*-0,045*	*0,732*

Spearman Rank correlation analysis

and preeclampsia [21-22]. Hogg et al., found no difference in homocysteine levels between preeclamptic patients and controls during the second trimester of pregnancy [23], but detected a difference in plasma homocysteine levels between severe preeclampsia group and the control group in the third trimester. They concluded that second-trimester homocysteine level was not a predictive parameter for the development of preeclampsia. Steegers et al., found that the risk of gestational hypertension, placental abruption and intrauterine fetal growth restriction increased with hyperhomosisteinemia but no relationship related to preeclampsia was present [24]. Zeeman et al., studied second-trimester homocysteine levels in patients with chronic hypertension to assess the risk of developing preeclampsia but found that homocysteine level was not useful as a predictive factor for developing preeclampsia [25]. Interestingly, Mignini et al., claimed that there was no relation between homocysteine levels and the development of preeclampsia, and also that hyperhomosisteinemia decreased the risk of preeclampsia [26].

Numerous studies showed the high homocysteine levels were related to folic acid deficiency [27-28]. Murphy et al., reported that folic acid supplementation had no effect on decreasing the high level of homocysteine [29]. On the other hand, in another study, folic acid supplementation decreased the level of homocysteine and risk of developing preeclampsia [30]. Braekke et al., found no difference in folic acid and vitamin B_{12} levels between the preeclamptic group and controls, but the level of plasma homocysteine may be predictive in determining fetal weight [31]. In our study, we found that mean folic acid levels in the preeclamptic and the control groups were 8.85±3.30 and 9.15±4.05, respectively (p>0.663), and there was no statistically significant difference. There was no significant difference in vitamin B_{12} level between the two groups (163.94±69.28 vs. 182.64±77.38; p>0.179).

Hyperhomocysteinemia has been associated with vascular damage, although whether it is cause or effect remains a matter of debate. Considering the heterogeneous pathophysiology of preeclampsia, no single screening test is sufficient for predicting preeclampsia. Early detection of the risk for preeclampsia may allow for the improvement of the pregnancy outcome by increasing patient surveillance or by initiating a therapeutic intervention. Based on our findings, it seems safe to conclude that plasma homocysteine levels increase significantly in patients with preeclampsia and that it is not correlated with the severity of preeclampsia. Further research is required in order to clarify the role of homocysteine in the etiology of preeclampsia.

Authors' contribution:
1. Fatih Şanlıkan – Concept, study design, analysis and interpretation of data, article draft, corresponding author.
2. Fatma Tufan – acquisition of data.
3. Ahmet Göçmen – revised article critically.
4. Ceyda Kabadayı – acquisition of data.
5. Erkan Şengül – acquisition of data.

Authors' statement
- This is to certify, that the publication will not violate the copyrights of a third party, as understood according to the Act in the matter of copyright and related rights of 14 February 1994, Official Journal 2006, No. 90, Clause 63, with respect to the text, data, tables and illustrations (graphs, figures, photographs);
- there is no 'conflict of interests' which occurs when the author remains in a financial or personal relationship which unjustly affects his/her actions associated with the publication of the manuscript;
- any possible relationship(s) of the author(s) with the party/parties interested in the publication of the manuscript are revealed in the text of the article;
- the manuscript has not been published in or submitted to any other journal.
- Source of financing: None

References

1. American College of Obstetricians and Gynecologists; Task Force on Hypertension in Pregnancy. Hypertension in pregnancy. Report of the American College of Obstetricians and Gynecologists' Task Force on Hypertension in Pregnancy. *Obstet Gynecol.* 2013,122, 1122-1131.
2. Roberts C, Algert C, Morris J, [et al.]. Hypertensive disorders in pregnancy: a population-based study. *Med J Aust.* 2005, 182, 332–335.
3. Hernandez-Diaz S, Toh S, Cnattingius S. Risk of pre-eclampsia in first and subsequent pregnancies: prospective cohort study. *BMJ.* 2009, 338:b2255.
4. Antoniades C, Antonopoulos AS, Tousoulis D, [et al.]. Homocysteine and coronary atherosclerosis: from folate fortification to the recent clinical trials. *Eur Heart J.* 2009, 30, 6-15.
5. Boushey CJ, Beresford SA, Omenn GS, Motulsky AG. A quantitative assessment of plasma homocysteine as a risk factor for vascular disease. *JAMA.* 1995, 274, 1049-1057.
6. Lin PY, Yang TH, Lin HG, Hu ML. Synergistic effects of S-adenosylhomocysteine and homocysteine on DNA damage in a murine microglial cell line. *Clin Chim Acta.* 2007, 379, 139-144.
7. López-Quesada E, Also-Rayo E, Vilaseca MA. Hyperhomocysteinemia during pregnancy as a risk factor of preeclampsia. *Med Clin (Barc).* 2003, 121, 35035-5.
8. Walker MC, Smith GN, Perkins SL, [et al.]. Changes in homocysteine levels during normal pregnancy. *Am J Obstet Gynecol.* 1999, 180, 660-664.
9. Refsum H, Fiskerstrand T, Guttormsen AB, Ueland PM. Assessment of homocysteine status. *J Inherit Metab Dis.* 1997, 20, 286–294.
10. Chesley LC. Plasma and red cell volumes during pregnancy. *Am J Obstet Gynecol.* 1972, 112, 440–450.
11. Obwegeser R, Hohlagschwandtner MHS. Homocysteine a pathophysiological cornerstone in obstetrical and gynecological disorders? *Human Reprod Update.* 1999, 5, 64–72.
12. Finkelstein J. Methionine metabolism in mammals. Effect of age, diet, and hormones on three enzymes of the pathway in rat tissues. *Arch Biochem Biophys.* 1972, 122, 583–590.
13. Kim MH, Kim E, Passen EL. Cortisol and estradiol: non genetic factors for hyperhomocystinemia. *Metabolism.* 1997, 46, 247–249.
14. Malinow MR, Rajkovic A, Druell PB, [et al.]. The relationship between maternal and neonatal umbilical cord plasma homocysteine suggests a potential role for maternal homocysteine in fetal metabolism. *Am J Obstet Gynecol.* 1998, 178, 228–233.
15. Lopez-Quesada E, Vilaseca MA, Lailla JM. Plasma total homocysteine in uncomplicated pregnancy and in preeclampsia. *Eur J Obstet Gynecol Reprod Biol.* 2003, 108, 45-49.
16. Makedos G, Papanicolaou A, Hitoglou A. Homocysteine, folic acid and B12 serum levels in pregnancy complicated with preeclampsia *Arch Gynecol Obstet.* 2007, 275, 121–124.
17. Powers HJ, Fraser R, Gibson AT. Antioxidants and pre-eclampsia. *Lancet.* 2000, 355, 64-65.
18. Var A, Yildirim Y, Onur E, [et al.]. Endothelial dysfunction in preeclamp-sia: increased homocysteine and decreased nitric oxide levels. *Gynecol Obstet Invest.* 2003, 56, 221–224.
19. Rajkovic A, Catalano PM, Malinow MR. Elevated homocyst(e)ine levels with preeclampsia. *Obstet Gynecol.* 1997, 90, 168-171.
20. Dodds L, Fell DB, Dooley KC, [et al.]. Effect of homocysteine concentration in early pregnancy on gestational hypertensive disorders and other pregnancy outcomes. *Clin Chem.* 2008, 54, 326-334.
21. Hietala R, Turpeinen U, Laatikainen T. Serum homocysteine at 16 weeks and subsequent preeclampsia. *Obstet Gynecol.* 2001, 97, 527-529.
22. Middeldorp S, van de Poel MH, Bank I, [et al.]. Unselected women with elevated levels of factor VIII: C or homocysteine are not at increased risk for obstetric complications. *Thromb Haemost.* 2004, 92, 787-790.
23. Hogg BB, Tamura T, Johnston KE, [et al.]. Secondtrimester plasma homocysteine levels and pregnancy-induced hypertension, preeclampsia, and intrauterine growth restriction. *Am J Obstet Gynecol.* 2000, 183, 805-809.
24. Steegers-Theunissen RP, Van Iersel CA, Peer PG, [et al.]. Hyperhomocysteinemia, pregnancy complications, and the timing of investigation. *Obstet Gynecol.* 2004, 104, 336-343.
25. Zeeman GG, Alexander JM, McIntire DD, [et al.]. Homocysteine plasma concentration levels for the prediction of preeclampsia in women with chronic hypertension. *Am J Obstet Gynecol.* 2003, 189, 574-576.
26. Mignini LE, Latthe PM, Villar J, [et al.]. Mapping the theories of preeclampsia: the role of homocysteine. *Obstet Gynecol.* 2005, 105, 411-425.
27. Dunn J, Title LM, Bata I, [et al.]. Relation of a common mutation in methylenetetrahydrofolate reductase to plasma homocysteine and early onset coronary artery disease. *Clin Biochem.* 1998, 31, 95-100.
28. Magne PM, Vollset SE. Homocysteine and Folate in Pregnancy. *Clin Chem.* 2004, 50, 1293-1295.
29. Murphy MM, Scott JM, McPartlin JM, Fernandez-Ballart JD. The pregnancy related decrease in fasting plasma homocysteine is not explained by folic acid supplementation, hemodilution, or a decrease in albumin in a longitudinal study. *Am J Clin Nutr.* 2002, 76, 614-619.
30. Wen SW, Chen XK, Rodger M, [et al.]. Folic acid supplementation in early second trimester and the risk of preeclampsia. *Am J Obstet Gynecol.* 2008,198, 45.e1-7.
31. Braekke K, Ueland PM, Harsem NK, [et al.]. Homocysteine, cysteine, and related metabolites in maternal and fetal plasma in preeclampsia. *Pediatr Res.* 2007, 62, 319-324.