Study of Serum Homocysteine, Folic Acid and Vitamin B₁₂ in Patients with Preeclampsia

Shahid A. Mujawar · Vinayak W. Patil ·
Rekha G. Daver

Received: 8 October 2010 / Accepted: 6 January 2011 / Published online: 19 January 2011
© Association of Clinical Biochemists of India 2011

Abstract The present study was carried out to evaluate the occurrence of association between homocysteine, folic acid and vitamin B₁₂ in patients with preeclampsia. Fifty preeclamptic patients from gynecology ward were studied for estimation of serum homocysteine, folic acid and vitamin B₁₂ over a period of October 2007 to June 2010. Serum homocysteine and folic acid, and vitamin B₁₂ were determined by means of Immulite 1000 analyzer. The statistical analysis of study group of preeclampsia compared with normotensive control group, showed significant alterations in serum homocysteine, folic acid and vitamin B₁₂ concentrations in preeclampsia. Inverse association between serum homocysteine and folic acid, and vitamin B₁₂ levels were observed in preeclampsia. The present study found hyperhomocysteinemia and deficiency of folic acid and vitamin B₁₂ along with increased blood pressure as a risk factor for cardiovascular disease (CVD) in preeclampsia.

Keywords Homocysteine · Folic acid · Vitamin B₁₂ · Preeclampsia

Introduction

Pregnancy induced hypertension may occur in about 3–10% of all pregnancies [1, 2]. It remain a major cause of perinatal and maternal morbidity and mortality world-wide, because of complications such as pre-eclampsia, eclampsia, fetal growth retardation, premature birth or abruptio placentae [3].

An increased concentration of total circulating homocysteine in serum is recognized as an independent risk factor for cardiovascular diseases (CVD) [4, 5]. Moreover, determinants of hyperhomocysteinemia, such as low concentrations of folic acid and vitamin B₁₂ involved in homocysteine metabolism are also associated with increased risk of vascular damage [6–8]. It is uncertain whether hyperhomocysteinemia per se or low concentrations of vitamin B₁₂ and folic acid are atherogenic factors that trigger vascular disease. The present study undertaken to determine the levels of serum homocysteine, folic acid and vitamin B₁₂ and their correlation in patients with preeclampsia.

Materials and Methods

This study was carried out at Department of Biochemistry, Grant Medical College and Sir J.J. Group of Government Hospitals, Mumbai. All participants completed a medical history form and provided informed consent. Fifty preeclamptic patients in the age group of 18–35 years were studied for estimation of serum total homocysteine, folic acid, and vitamin B₁₂ over a period of January 2008 to June 2010. Control group consist of 50 healthy normotensive pregnant subjects of identical age without any disease were enrolled.

S. A. Mujawar · V. W. Patil
Department of Biochemistry, Grant Medical College
and Sir J.J. Group of Government Hospitals, Mumbai 400008,
Maharashtra, India

R. G. Daver
Department of Obstetrics and Gynecology, Grant Medical
College and Sir J.J. Group of Government Hospitals,
Mumbai 400008, Maharashtra, India

S. A. Mujawar (✉)
Flat No-30, 7th Floor, Swstik-4, Sir J.J. Hospital Campus,
Byculla, Mumbai 400008, Maharashtra, India
e-mail: akbarson4@gmail.com

Inclusion Criteria

Patients with symptoms and signs suggestive of eclampsia supported by laboratory investigations. And with normal renal and liver function tests were included in the study.

Exclusion Criteria

Patients having use of medications (therapy involving S-adenosyl-methionine, carbamazepine, phenytoin, 6-azauridine, xanthopterin, antifolic acids, anticonvulsant agents, tamoxifen, and theophylline), cancer, anemia, systemic illness and those with major illness were excluded from study. The Institutional Ethical Committee at the Grant Medical College and Sir J.J. Group of Government Hospitals, Mumbai, India, approved the study.

Blood Sample Collection

Venous blood samples were collected in test tube with aseptic precautions. After 2 h of collections sample was centrifuged at 3000 rpm for 5 min. Serum was separated and collected in polythene tube with cork. The sera with no sign of hemolysis used for the analysis of total circulating homocysteine, folic acid, vitamin B₁₂ and thyroid function.

Biochemical Analysis

Serum homocysteine concentration was measured by competitive chemiluminescent enzyme immunoassay method [9]. Serum folic acid concentration was investigated by boil competitive, liquid phase, ligand labeled, and protein binding chemiluminescent assay method [10]. Serum vitamin B₁₂ concentration was evaluated by solid phase, competitive chemiluminescent assay method [11]. We used fully automated enzyme amplified chemiluminescent immuno assay based Immulite 1000 analyzer. Measurement of these blood parameters by using commercial kits from Siemens Medical Solutions Diagnostics, Los Angeles, CA, USA. Hyperhomocysteinemia was defined as a serum homocysteine concentration greater than 16 µmol/l.

Statistical Analysis

Numerical variables were reported in terms of mean and standard deviation. Statistical analysis of results was done by normal distribution 'Z' test. In this analysis, variables showing *P*-value less than 0.05 and 0.001 were considered to be statistically significant and highly significant, respectively. Pearson correlation test was used to test correlation.

Results

Demographic data of preeclamptic patients such as mean age of preeclampsia patients was showed significant fall ($P < 0.05$) whereas body mass index (BMI), systolic blood pressure (SBP) and diastolic blood pressure (DBP) are highly significant increase ($P < 0.001$) as compared with control group (Table 1).

Table 2 depicts changes in serum profile when control group was compared with study group of preeclampsia. As can be seen, significant increase ($P < 0.001$) were observed in serum homocysteine whereas, folic acid and vitamin B₁₂ levels showed significant decrease ($P < 0.001$).

A negative and significant correlation was observed between serum homocysteine compared with vitamin B₁₂ whereas an inverse and insignificant relationship was found between serum homocysteine and folic acid in preeclampsia. (Table 3).

Table 1 Demographic data in controls and preeclamptic patients

Parameters	Control group (<i>n</i> = 50)	Preeclampsia group (<i>n</i> = 50)	<i>P</i> -value
Age (years)	26.4 ± 4.48	23.6 ± 4.16	0.0013*
Gestation age (weeks)	23.8 ± 10.2	32.4 ± 6.05	0.0001**
BMI (kg/m ²)	21.9 ± 4.78	29.9 ± 4.81	0.0001**
SBP (mm of Hg)	122 ± 6.45	161 ± 9.39	0.0001**
DBP (mm of Hg)	81.8 ± 4.78	94.9 ± 7.46	0.0001**

The results were compared between normotensive pregnant group as a control and preeclampsia group. The values are presented in mean ± S.D. * $P < 0.05$, ** $P < 0.001$

Table 2 Serum total homocysteine, folic acid and vitamin B₁₂ in controls and preeclamptic patients

Biochemical parameters	Control group (<i>n</i> = 50)	Preeclampsia group (<i>n</i> = 50)	<i>P</i> -value
Homocysteine (µmol/l)	8.19 ± 1.68	16.4 ± 3.26	0.0001**
Folic acid (ng/ml)	10.9 ± 2.13	9.71 ± 1.22	0.0002**
Vitamin B ₁₂ (pg/ml)	391 ± 105	321 ± 64.9	0.0001**

The results were compared between normotensive pregnant group as a control and preeclampsia group. The values are presented in mean ± S.D. * $P < 0.05$, ** $P < 0.001$

Table 3 Correlation of total homocysteine and folic acid, and vitamin B₁₂ in preeclamptic patients

Parameters	95% CI of 'r'	'r' Value	<i>P</i> -value
Folic acid	−0.449 to 0.088	−0.196	0.174 ^{NS}
Vitamin B ₁₂	−0.561 to −0.062	−0.335	0.018*

CI Confidence interval, *r* Correlation coefficient, * $P < 0.05$, NS Not significant

Discussion

Our finding suggests that levels of serum homocysteine, folic acid and vitamin B₁₂ are altered in preeclampsia patients than in age-matched normotensive pregnant control subjects. In preeclampsia patients, weight, BMI, SBP and DBP MBP showed significant increase. Importance of these parameters has been recognized estimating cardiovascular disease risk factor due to their positive association with hypertension [12].

The present study shows that there was significant hyperhomocysteinemia and deficiency of folic acid and vitamin B₁₂ patients with preeclampsia. Hyperhomocysteinemia in such preeclamptic patients were found in our study might be due to modulation in homocysteine metabolism, which corroborates with the work of Walker et al. [13], Hogg et al. [14], Vollset et al. [15]. About more than one-half of the cases (54%) had hyperhomocysteinemia in study group of preeclamptic patients in our study. These prospective studies with rather small cohorts of patients with preeclampsia have shown an independent association between elevated serum homocysteine level and cardiovascular complications.

Several factors may increase homocysteine levels in women with preeclampsia. Metabolism in the kidney is the major route by which homocysteine is cleared from plasma and this route of elimination may be affected by preeclamptic changes in the kidney [16]. Several studies have demonstrated serum concentrations of homocysteine increases in preeclampsia [13–15]. These studies support our results.

In our study, the levels of vitamins B₁₂ and folic acid were significantly lowered in the preeclamptic as compared to control groups suggesting that there were the vitamin deficiencies. Carmel R found that differences in folic acid concentrations between preeclamptic and normal pregnant women [17]. Similarly, in a systematic review by Mignini et al., folic acid and vitamin B₁₂ concentrations were lower in preeclamptic women when compared with those of normotensive women [18]. In another study, there was no difference in folic acid and vitamin B₁₂ levels between pooled normal and preeclamptic groups but these levels were significantly lower in patients with the 677 CT mutation of MTHFR [19].

The serum homocysteine was found to have negative and insignificant correlation with serum folic acid in preeclamptic patients. Their correlation coefficient (r) of -0.196 ($P = 0.174$) and 95% CI of ' r ' ranged from -0.449 to $+0.088$ (Fig. 1). A good, negative and statistically significant correlation ($r = -0.335$ and $P < 0.05$) was found between serum homocysteine and vitamin B₁₂ in preeclampsia. Their 95% CI of ' r ' ranged from -0.561 to -0.062 (Fig. 2). In present literature, there are two pathways by which homocysteine metabolized: remethylation

Fig. 1 The correlation between serum homocysteine and folic acid in preeclampsia

Fig. 2 The correlation between serum homocysteine and vitamin B₁₂ in preeclampsia

and transsulfuration. Folic acid and vitamin B₁₂ are required for the remethylation of homocysteine to methionine; vitamin B₆ is required for the transsulfuration of homocysteine to cysteine [20]. A good correlation between serum homocysteine and folic acid, and vitamin B₁₂ levels observed in our study support this view.

From the above discussion we can assume that biochemical screening such as homocysteine, folic acid, vitamin B₁₂ are of paramount importance in preeclampsia. The inverse relation between homocysteine and folic acid, and vitamin B₁₂ indicates that severity associated with metabolic disturbances in preeclampsia that can be contributed to CVD. On the other hand, there is an absolute need for large studies designed to answer the question as to whether hyperhomocysteinemia and vitamin B deficiency are associated with increased risk for CVD and whether therapy of these disorders might influence cardiovascular mortality. Further studies should help define the role of genetic

polymorphism in enzymes of homocysteine, folic acid, vitamin B₁₂ metabolism and their role in preeclampsia.

References

1. Saftlas AF, Olson DR, Franks L, Atrash HK, Pokras R. Epidemiology of preeclampsia and eclampsia in the United States 1979–86. *Am J Obstet Gynecol.* 1990;163:460–5.
2. Barron WM, Murphy MB, Lindheimer MD. Management of hypertension during pregnancy. In: Laragh GH, Brenner BM, editors. *Hypertension: pathophysiology, diagnosis and management.* Raven: New York; 1990. p. 1809–27.
3. National high blood pressure education program working group report on high blood pressure in pregnancy. *Am J Obstet Gynecol* 1990; 163:1691–1712.
4. Nygard O, Nordrehaug JE, Refsum H, Ueland PM, Fastad M, Vollset SE. Plasma homocysteine levels and mortality in patients with coronary artery disease. *N Engl J Med.* 1997;337:230–6.
5. Wald DS, Law M, Morris JK. Homocysteine and cardiovascular disease: evidence on causality from a meta-analysis. *Br Med J.* 2002;325:1202–6.
6. Voutilainen S, Rissanen TH, Virtanen J, Lakka TA, Salonen JT. Low dietary folic acid intake is associated with an excess incidence of acute coronary events: the Kuopio ischemic heart disease risk factor study. *Circulation.* 2001;103:2674–80.
7. Klerk M, Verhoef P, Clarke R. MTHFR studies collaboration group. MTHFR 677C T polymorphism and risk of coronary heart disease: a meta-analysis. *J Am Med Assoc.* 2002;288:2023–31.
8. Durga J, Bots ML, Schouten EG, Kok FJ, Verhoef P. Low concentrations of folic acid, not hyperhomocysteinemia, are associated with carotid intima-media thickness. *Atherosclerosis.* 2005;179:285–92.
9. Ueland PM, Refsum H, Stabler SP, Malinow MR, Andersson A, Allen RH. Total homocysteine in plasma or serum: methods and clinical applications. *Clin Chem.* 1993;39:1764–79.
10. McNeely MDD. Folic acid assay. In: Kaplan LA, Pesce AJ, editors. *Clinical chemistry.* St. Louis: CV Mosby; 1984. p. 1402–6.
11. Allen RH. Clinical role and current status of serum cobalamin (vitamin B₁₂) assays. *Ligand Quarterly* 1981 Fall; 4(3):37–44.
12. Pi-Sunyer FX. Medical hazards of obesity. *Ann Intern Med.* 2000;160:2847–53.
13. Walker MC, Smith GN, Perkins SL, Keely EJ, Garner PR. Changes in homocysteine levels during normal pregnancy. *Am J Obstet Gynecol.* 1999;180:660–4.
14. Hogg BB, Tamura T, Johnston KE, DuBard MB, Goldenberg MA, Goldenberg RL. Second-trimester plasma homocysteine levels and pregnancy-induced hypertension, preeclampsia, and intrauterine growth restriction. *Am J Obstet Gynecol.* 2000;183:805–9.
15. Vollset SE, Refsum H, Irgens LM, Emblem BM, Tverdal A, Gjessing HK, et al. Plasma total homocysteine, pregnancy complications, and adverse pregnancy outcomes: the Hordaland homocysteine study. *Am J Clin Nutr.* 2000;71:962–8.
16. Bostom AG, Lathrop L. Homocysteinemia in end-stage renal disease: prevalence, etiology, and potential relationship to arteriosclerotic outcomes. *Kidney Int.* 1997;52:10–20.
17. Carmel R. Ethnic and racial factors in cobalamin metabolism and its disorders. *Semin Hematol.* 1999;36:88–100.
18. Mignini L, Latthe P, Villar J, Kilby M, Carroli G, Khan K. Mapping the theories of preeclampsia: the role of homocysteine. *Obstet Gynecol.* 2005;105:411–25.
19. Lachmeijer AM, Arngrimsson R, Bastiaans EJ, Pals G, ten Kate LP, de Vries JIP. Mutation in the gene for methylenetetrahydrofolate reductase, homocysteine levels and vitamin status in women with history of preeclampsia. *Am J Obstet Gynecol.* 2001;184:394–402.
20. Finkelstein JD. Methionine metabolism in mammals. *J Nutr Biochem.* 1990;1:228–37.