Measurement of Homocysteine Levels in Pregnant Women with and Without Preeclampsia

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Original Research Article

ABSTRACT

Introduction: Homocysteine is associated with endothelial dysfunction and cardiovascular disease, and elevated concentrations of homocysteine have been found in preeclampsia. The aim of this study was to determine homocysteine levels in pregnant women with and without preeclampsia.
Methods: This descriptive study conducted on pregnant women with preeclampsia (n = 39) and controls (n = 43) was evaluated by the convenience sampling and data were collected through a questionnaire and paraclinical findings. In the present study, 5cc blood samples were taken from the patients after 8 hours of fasting and sent to the laboratory to determine homocysteine and related metabolites levels. Statistical analyses were done using IBM-SPSS 25.0 and Chi-square test was used for data analysis.
Results: The moderate concentrations of homocysteine and uric acid were significantly higher than the control group in maternal plasma with preeclampsia (0.0001).

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Conclusion: We found that the blood homocysteine levels were significantly higher in the preeclampsia group compared with the Controls group. Measuring uric acid and blood homocysteine levels in pregnancy may be helpful as diagnostic tests in the early detection of high risk individuals.

1. INTRODUCTION

Preeclampsia is defined as the combination of pregnancy-induced hypertension and proteinuria in the second half of the pregnancy [1] that can affect almost all organs and cause morbidity and mortality in the mother and fetus [2]. The syndrome may have both short- and long-term consequences, including cardiovascular disease later in life [3]. Gestational hypertension disorders occur in 3–10% of all pregnancies, and among the types of hypertension, preeclampsia syndrome is considered as the most dangerous condition and is the second leading cause of maternal death in developing countries [4]. Preeclampsia often affects young women [5] and influenced by race and ethnicity. Other influencing factors include environmental impacts such as obesity, high salt intake, alcohol and stress, socioeconomic status, and even seasonality [6,7].

The hypotheses of the mechanism of preeclampsia include placental origin, immunological origin, genetic susceptibility, thrombophilia, endothelial injury, increase of Oxygen-derived free radicals, and nutritional deficiency [6].

High circulating levels associated with cardiovascular disease [8,9]. In uncomplicated pregnancies, high maternal homocysteine concentration has been associated with low birth weight in offspring [10] of homocysteine can cause endothelial dysfunction [11] and elevated plasma concentrations of homocysteine is [12]. Elevated maternal concentrations of homocysteine have been reported in preeclampsia [13-15].

Homocysteine is one of the factors that is associated with diseases such as spontaneous abortion and placental disorders. In addition, a decrease in folic acid and vitamin B12 concentrations increases homocysteine and, consequently, increases the risk of vascular injury [16-18]. Homocysteine is a sulfur-containing amino acid derived from the removal of the methyl group in methionine, an essential amino acid. Folate (B9) and vitamin B12 are homocysteine re-methylation cofactors [19]. Some researchers have reported hyperhomocysteinemia as a major cause of arterial and venous thrombosis and believed that variable folate levels may play a key role in increasing or decreasing blood homocysteine. Studies have shown that prophylactic folic acid supplementation may be recommended as an effective factor in preventing hypertensive disorders in pregnancy [19]. Assay of effective related factors and screening women at risk for preeclampsia would increase the potential for strategies of prevention [20]. Changes in homocysteine levels in each trimester of pregnancy have shown that its highest decrease at gestational age of 20-28 weeks is about 4.3 µmol/L and the highest level is about 5.5 µmol / l at 36-42 weeks [21]. The aim of this study was to investigate maternal concentrations of total homocysteine in pregnant women with and without preeclampsia.

2. MATERIALS AND METHODS

2.1 Study Design

This descriptive study was conducted in Ali Ibn Abi Talib educational hospital and a tertiary center of Sistan and Baluochestan province in Zahedan, Iran from may 2017 to august 2019. In this study, we included women with preeclampsia (N = 39) and uncomplicated pregnancies (controls, n = 43), all the women were fasting and were not in active labor, and none had rupture of membranes or signs of infection. The sampling method was convenience sampling. Furthermore, data were collected through a questionnaire and paraclinical results.

2.1.1 Inclusion criteria

Women with age 15-45 years, single pregnancy, natural hemoglobin, lack of chronic hypertension, no history of thrombophilia no history of lupus or underlying disease.

2.1.2 Exclusion criteria

Women with a history of chronic hypertension, smoking or drug abuse, overt or gestational

diabetes, kidney disease, hemoglubinopathies, megaloblastic anemia, seizure, prior significant illness, personal or familial history of deep vein thrombosis, and/or vitamin deficiency as well as those receiving any anti-folate drugs (Antiepileptics and methotrexate).

2.2 Biologic Samples

After obtaining informed consent from patients, five cc of venous blood was taken after 8 hours fasting and before delivery and sent to laboratory for determination of homocysteine level. The control group was also selected from pregnant mothers referred to Ali Ibn Abi Talib Hospital who had normal blood pressure and did not have proteinuria in the dipstick test. All patients were told that this procedure had no consequences and it will not impose a cost on the patient. People were asked exactly the history of taking supplements. The history of the supplements was taken exactly from the individuals.

2.3 Data Collection

Also, the definition of preeclampsia in this study is based on blood pressure ≥140/90 mm Hg and proteinuria ≥+2 in a dipstick test or ≥0.3 g in 24-hour urine collection. Venous blood samples were collected in test tubes containing clot activator, immediately stored on ice and—one hour after collection—centrifuged at 4000 rpm for 10 minutes. Plasma was separated and stored at −80°C through the hour to be analyzed. Demographic data of patients and laboratory results were recorded in a pre-designed information form and finally entered for statistical analysis.

2.4 Biochemical Analyses

Homocysteine concentration was measured in EDTA plasma by high-performance liquid chromatography (HPLC), using a homocysteine kit from Bio-Rad (Bio-Rad Laboratories, Munich, Germany) essentially as described by the manufacturer. Other related metabolites levels were measured with electrochemiluminescence using kits from Roche Company, Germany.

2.5 Statistical Analysis

Data were analyzed using descriptive statistics (mean), independent t-test and chi-square. *SPSS V.25* was used to apply for statistical analysis. Statistical significance was assessed at the 5% level.

3. RESULTS

The means age were 28.86±5.61 and 26.93 ±6.42 years in the preeclampsia and controls groups, respectively (P = 0.2), there is no significant difference between the mean between two groups. Patient demographic data are shown in Table 1. The mean weight in the controls and preeclampsia groups was 70.03 ±17.25 kg and 72.20 ± 11.92 kg, respectively (P=0.6). Considering the weight of pregnant mothers, the difference was not found to be significant between the two groups and the weight of the two groups was almost the same. The gestational age was 38 ±1.4 and 36± 3.4 weeks in the controls and preeclampsia groups, respectively. There was a statistically significant difference between the preeclampsia group and the control group (P=0.03). Therefore, pregnant mothers with preeclampsia had lower gestational age at the time of hospitalization. The number of previous pregnancies was evaluated in both groups. The controls and Preeclampsia group had 3.5 ±2.2 births and 3.3 ±2.1 births, respectively. No statistically significant difference was found between the two groups in *pregnancy numbers* (p=0.6). The mean systolic and diastolic blood pressure in the Preeclampsia group were 155.66 and 92.66 mmHg, respectively, and these values were 113.33 and 78.66 in healthy pregnant mothers, respectively, indicating a higher blood pressure in pregnant mothers with preeclampsia (0.0001).

Table 1. Demographic data in controls and preeclampsia group

Parameters	Preeclampsia group (n=39)	Controls (n=43)	P-value
Age (year)	26.93 ±5.61	28.86 ±6.42	0.2
Weight(kg)	72.20 ± 11.92	70.03 ±17.25	0.6
Gestational age (week)	36.16 ± 3.4	38 ±1.4	0.03
Gravida	3.3 ±2.1	3.5 ±2.2	0.6
Systolic blood pressure (mmHg)	155.66 ± 12.06	92.66 ± 6.85	<0.001
Diastolic blood pressure (mmHg)	113.33 ± 5.69	78.66 ± 5.07	<0.001

Table 2. Plasma concentrations of biochemical variables in preeclampsia and control groups

Biochemical Parameters	Preeclampsia (n=39)	Control group (n=43)	P-value
Homocysteine (µmol/L)	8.9 ± 5.32	3.9 ± 1.62	0.0001
Uric acid (ng/mL)	5.7 ±1.4	4.2 ±1.2	<0.001
Platelet (µg /ml)	177.36 ± 84.35	193.33 ±41.2	0.3
AST (IU/L)	22.9±4.83	20.7± 5.22	0.97
ALT (IU/L)	14.53±5.07	12.53±3.59	0.33
Lactate dehydrogenase(IU/L)	353.6±110.54	340.41±109.21	0.65

Comparison of homocysteine levels in pregnant women with and without preeclampsia referred to the hospital was evaluated and the homocysteine level was 3.9 ±1.6 µmol in the Controls and 8.9 ±5.3 µmol in the Preeclampsia group. Furthermore, homocysteine levels in Preeclampsia group were higher than Control group that this difference was statistically significant (0.0001).

Uric acid status was evaluated in both preeclampsia and non-preeclamptic groups. Uric acid content in the Controls group was 4.2 ±1.2 and in the Preeclampsia group was 5.7 ±1.4 mg /dL. Statistically significant difference was observed between the two groups (P = 0.0001), where uric acid in pregnant women with preeclampsia is much higher than in healthy pregnant mothers. Platelet counts in the two groups were 193.33 ±41.2 and 177.36 ± 84.35, respectively. The number of platelets in the Preeclampsia group was found to be lower as compared to the Controls group, but this difference was not statistically significant (P = 0.3).

Assessment of aspartate amino transferase (AST) revealed that its levels were 22.9±4.83 IU/L in the Controls and 20.7± 5.22 IU/L in Preeclampsia group (p= 0.09). Aminotransferase was determined to be 12.53±3.59 IU/L and 14.53±5.07 IU/L in controls and preeclampsia group, respectively (P = 0.13). Lactate dehydrogenase status was evaluated in Controls and Preeclampsia group and it was found that the level of lactate dehydrogenase was 340.41±109.21U/L in the healthy group, whereas this amount was found to be 353.6±110.54 IU /L in the patient group. There was no statistically significant difference between the two groups (P = 0. 65).

4. DISCUSSION

Preeclampsia occurs frequently in pregnancy and poses several risks to the mother and fetus [22]. Some studies have, however, reported an increased risk of preeclampsia associated with higher homocysteine concentrations [23-27]. In this study, we found that homocysteine levels in Preeclampsia group were higher than the control group and this difference was statistically significant. Platelet، AST ،ALT and Lactate dehydrogenase concentrations did not differ between the preeclampsia and control groups. In the preeclampsia group, we found an inverse association between homocysteine and Systolic and Diastolic blood pressure.

A similar study by Shohreh et al. [28] reported that the mean serum levels of homocysteine in the third trimester of pregnancy were significantly higher in the preeclampsia group than in the normal group. Amniotic fluid homocysteine levels were also significantly higher in the preeclamptic group [28].

In normal pregnancies also, homocysteine concentrations are lower in the second than in the first trimester and increase toward nonpregnant values in the second half of the third trimester [24]. The etiology of the higher homocysteine plasma concentrations in preeclamptic pregnancies is not known. Studies of methylenetetrahydrofolate reductase (MTHFR) polymorphisms, folate, and vitamin B12 have failed to prove these to be of significant impact [29,30].

Amino acids are transported actively across the placenta, as indicated by higher fetal than maternal concentrations for most amino acids [31]. Studies of animal and human models report decreased placental transport of amino acids to the fetus in situations of growth restriction, but not always reduced fetal concentrations [31,32]. One study reported increased maternal amino acid concentrations in preeclampsia compared with controls [33]. It has been hypothesized that altered amino acid metabolism in pregnancy is driven by fetal requirements through unknown mechanisms [34].

In the present study, blood uric acid levels were significantly correlated with the incidence of

preeclampsia, which was consistent with Wakwe and Abudu 1999 in which a significant relationship was found between serum uric acid levels and the incidence of preeclampsia [25].

Another study by Singh et al. [35] showed that hyperhomocysteinemia was caused by endothelial dysfunction of the arteries and was involved in placental infarction, Placental abruption, and repetitive miscarriage, result in restriction of fetal growth and neurological disorders by crossing the placenta [35]. A study by Hoque et al. [36] concluded that homocysteine increased significantly in preeclampsia, but this increase was more dominant in eclampsia, consistent with our study [36].

A limitation of our study is that the sample size is small; another is that the median gestational age differs between preeclampsia and controls. It is difficult to match for gestational age between the often prematurely delivered preeclamptic patients and women with uncomplicated deliveries. Several studies have shown that homocysteine concentrations are lower in early third trimester than at term; thus, the differences demonstrated in our study between preeclamptics and controls are probably underestimated.

Since previous studies have shown that early pregnancy hyperhomocysteinemia can increase the incidence of preeclampsia in later stages of pregnancy, it is advisable to measure serum homocysteine levels during the pregnancy and even prior to conception. In point of view of the vitamin deficiencies associated with hyperhomocysteinemia such as the deficiency of folate and vitamins B6 and B12, the above supplements are recommended during pregnancy and before. These results could prove useful for further research regarding the causes and consequences of elevated homocysteine in preeclampsia, as well as in potential intervention studies

5. CONCLUSION

The authors found that the blood homocysteine levels were significantly higher in the preeclampsia group compared with the Controls group. Hence, monitoring this item in high-risk pregnant women over 20-28 weeks of gestational age could be of benefit for early detection of preeclampsia. Measuring uric acid and blood homocysteine levels in pregnancy may be helpful as early tests in the early detection of high risk individuals.

CONSENT AND ETHICAL APPROVAL

The study was obtained and general guidelines of ethics in medical science research were followed. The project was approved by the research department of the Faculty of Medicine. Informed and written consent was obtained from patients.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Roberts JM, Redman CG. Pre-eclampsia: More than pregnancy-induced hypertension. Lancet (British edition). 1993;341(8858):1447-51.
2. Saftlas AF, Olson DR, Franks AL, Atrash HK, Pokras R. Epidemiology of preeclampsia and eclampsia in the United States, 1979-1986. American Journal of Obstetrics and Gynecology. 1990;163(2): 460-5.
3. Sibai B, Dekker G, Kupferminc M. Pre-eclampsia. The Lancet. 2005;365(9461): 785-99.
4. Barron W, Murphy M, Lindheimer M. Management of hypertension during pregnancy. Hypertension: Pathophysiology, diagnosis and management Raven: New York. 1990:1809-27.
5. Hall GH, Noble WL, Lindow SW, Masson EA. Long-term sexual co-habitation offers no protection from hypertensive disease of pregnancy. Human Reproduction. 2001;16(2):349-52.
6. Stiefel P, Miranda ML, Bellido LM, Luna J, Jiménez L, Pamies E, et al. Genotype of the CYBA promoter− 930A/G, polymorphism C677T of the MTHFR and APOE genotype in patients with hypertensive disorders of pregnancy: An observational study. Medicina Clinica. 2009;133 (17):657-61.
7. Nygård O, Nordrehaug JE, Refsum H, Ueland PM, Farstad M, Vollset SE. Plasma homocysteine levels and mortality in patients with coronary artery disease. New

England Journal of Medicine. 1997;337(4):230-7.

8. Collaboration HS. Homocysteine and risk of ischemic heart disease and stroke: A meta-analysis. Jama. 2002;288(16):2015-22.

9. El-Khairy L, Ueland PM, Refsum H, Graham IM, Vollset SE. Plasma total cysteine as a risk factor for vascular disease: The European Concerted Action Project. Circulation. 2001;103(21): 2544-9.

10. Murphy MM, Scott JM, Arija V, Molloy AM, Fernandez-Ballart JD. Maternal homocysteine before conception and throughout pregnancy predicts fetal homocysteine and birth weight. Clinical Chemistry. 2004;50(8):1406-12.

11. Wilson KM, Lentz SR, editors. Mechanisms of the atherogenic effects of elevated homocysteine in experimental models. Seminars in vascular medicine: Copyright© 2005 by Thieme Medical Publishers, Inc., 333 Seventh Avenue, New; 2005.

12. Malinow MR, Rajkovic A, Duell PB, Hess DL, Upson BM. The relationship between maternal and neonatal umbilical cord plasma homocyst (e) ine suggests a potential role for maternal homocyst (e) ine in fetal metabolism. American Journal of Obstetrics and Gynecology. 1998; 178(2):228-33.

13. Powers RW, Evans RW, Majors AK, Ojimba JI, Ness RB, Crombleholme WR, et al. Plasma homocysteine concentration is increased in preeclampsia and is associated with evidence of endothelial activation. American Journal of Obstetrics and Gynecology. 1998;179(6):1605-11.

14. Raijmakers MT, Zusterzeel PL, Steegers EA, Hectors MP, Demacker PN, Peters WH. Plasma thiol status in preeclampsia. Obstetrics & Gynecology. 2000;95(2):180-4.

15. El-Khairy L, Vollset SE, Refsum H, Ueland PM. Plasma total cysteine, pregnancy complications, and adverse pregnancy outcomes: The hordaland homocysteine study. The American Journal of Clinical Nutrition. 2003;77(2):467-72.

16. Nadafi M, Mohammad Hosseini S, Afrasiabyfar A, Momeni E, Malekzadeh G. Association of homocysteine, vitamin and blood factors with preeclampsia in pregnant women. Armaghane Danesh. 2010;15(2):171-80.

17. Fu ZM, Ma ZZ, Liu GJ, Wang LI, Guo Y. Vitamins supplementation affects the onset of preeclampsia. Journal of the Formosan Medical Association. 2018;117(1):6-13.

18. Craig SA. Betaine in human nutrition. The American Journal of Clinical Nutrition. 2004;80(3): 539-49.

19. Hoffman ML, Scoccia B, Kurczynski TW, Shulman LP, Gao W. Abnormal folate metabolism as a risk factor for first-trimester spontaneous abortion. The Journal of Reproductive Medicine. 2008;53(3):207-12.

20. Dodds L, Fell DB, Dooley KC, Armson BA, Allen AC, Nassar BA, et al. Effect of homocysteine concentration in early pregnancy on gestational hypertensive disorders and other pregnancy outcomes. Clinical Chemistry. 2008;54(2):326-34.

21. Walker MC, Smith GN, Perkins SL, Keely EJ, Garner PR. Changes in homocysteine levels during normal pregnancy. American Journal of Obstetrics and Gynecology. 1999;180(3):660-4.

22. Daly S, Cotter A, Molloy AE, Scott J, editors. Homocysteine and folic acid: implications for pregnancy. Seminars in vascular medicine: Copyright© 2005 by Thieme Medical Publishers, Inc., 333 Seventh Avenue, New; 2005.

23. Baksu A, Taskın M, Goker N, Baksu B, Uluocak A. Plasma homocysteine in late pregnancies complicated with preeclampsia and in newborns. American Journal of Perinatology. 2006;23(01):031-6.

24. Herrmann W, Isber S, Obeid R, Herrmann M, Jouma M. Concentrations of homocysteine, related metabolites and asymmetric dimethylarginine in preeclamptic women with poor nutritional status. Clinical Chemistry and Laboratory Medicine (CCLM). 2005;43(10):1139-46.

25. Wakwe V, Abudu O. Estimation of plasma uric acid in Pregnancy Induced Hypertension (PIH). Afr J Sci. 1999;155-8.

26. Maged AM, Saad H, Meshaal H, Salah E, Abdelaziz S, Omran E, et al. Maternal serum homocysteine and uterine artery Doppler as predictors of preeclampsia and poor placentation. Archives of Gynecology and Obstetrics. 2017;296(3):475-82.

27. Cheng PJ, Huang SY, Su SY, Hsiao CH, Peng HH, Duan T. Prognostic value of cardiovascular disease risk factors measured in the first-trimester on the

severity of preeclampsia. Medicine. 2016; 95(5).

28. Shohreh M, Negin R, Z M, W F, SA N H. Center for homocysteine services in amniotic Massage and Preeclampsia blood transfusion and control group. Journal of Kermanshah University of Medical Sciences. 2012;7(16):532-7.

29. Long S, Goldblatt J. MTHFR genetic testing: Controversy and clinical implications. Australian Family Physician. 2016;45(4):237.

30. Mignini LE, Latthe PM, Villar J, Kilby MD, Carroli G, Khan KS. Mapping the theories of preeclampsia: The role of homocysteine. Obstetrics & Gynecology. 2005;105(2): 411-25.

31. Cetin I, Ronzoni S, Marconi AM, Perugino G, Corbetta C, Battaglia FC, et al. Maternal concentrations and fetal-maternal concentration differences of plasma amino acids in normal and intrauterine growth-restricted pregnancies. American Journal of Obstetrics and Gynecology. 1996;174 (5):1575-83.

32. Regnault T, Friedman J, Wilkening R, Anthony R, Hay Jr W. Fetoplacental transport and utilization of amino acids in IUGR — A review. Placenta. 2005;26:S52-S62.

33. Evans R, Powers R, Ness R, Cropcho L, Daftary A, Harger G, et al. Maternal and fetal amino acid concentrations and fetal outcomes during pre-eclampsia. Reproduction-Cambridge. 2003;125(6):7 85-90.

34. Holmes VA, Wallace JM, Alexander HD, Gilmore WS, Bradbury I, Ward M, et al. Homocysteine is lower in the third trimester of pregnancy in women with enhanced folate status from continued folic acid supplementation. Clinical Chemistry. 2005;51(3):629-34.

35. Singh U, Gupta H, Singh R, Shukla M, Singh R, Mehrotra S, et al. A study of changes in homocysteine levels during normal pregnancy and pre-eclampsia. Journal of the Indian Medical Association. 2008;106(8):503-5.

36. Hoque M, Bubult M, Islam N, Ferdousi M. Comparative Study on serum levels of homocysteine, folic acid and vitamin B12 in normal pregnants complicated with acute preeclampsia. Bangladesh Med Res Caun C Bull. 2008;1 (34):16-20.