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Original Research Article

Changes in homocysteine levels during normal pregnancy and preeclampsia and its relation with oxidative stress

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ABSTRACT

Background: Preeclampsia is a pregnancy specific disorder characterised by vasospasm and endothelial dysfunction. One of the most favoured hypotheses is the endothelial dysfunction secondary to the peroxidation of membrane lipids resulting in altered vascular reactivity, loss of vascular integrity and activation of the coagulation cascade. Elevated circulating homocysteine is a risk factor for endothelial dysfunction and vascular diseases and is found to be associated with preeclampsia. Hyperhomocysteinemia increases the risk of atherosclerosis through a mechanism involving oxidative damage. Malondialdehyde, (MDA) a metabolite of lipid peroxides detectable in plasma is used as an indicator of lipid peroxidation.
Methods: The present study was undertaken to find out the alterations in the circulating levels of serum homocysteine and malondialdehyde (MDA) in normal pregnancy and preeclampsia when compared to normal nonpregnant women. The case control study was conducted by taking a statistical sample size of 30 subjects (18- 35 years) in each group. Data were analyzed using ANOVA. Significance level was fixed at $p<0.05$.
Results: The mean serum levels of homocysteine were higher in preeclampsia patients than normal pregnant women. The mean serum levels of MDA in preeclampsia patients were higher than that of normal pregnant women.
Conclusions: The increased homocysteine levels in preeclampsia results in endothelial dysfunction and vasospasm. Also oxidative stress plays an important role in the pathogenesis of preeclampsia. Thus identifying the risk factors and aggressive management may prove to be beneficial in these women.

Keywords: Homocysteine, Malondialdehyde, Oxidative stress, Preeclampsia

INTRODUCTION

Preeclampsia is one of the most common pregnancy complications causing high morbidity and mortality for both mother and fetus especially in developing countries. Although the exact cause of preeclampsia is still unknown, the basic pathology lies in the endothelial dysfunction and intense vasospasm.[1] Homocysteine is an amino acid formed during demethylation of dietary methionine. Elevated blood levels of homocysteine are now recognized as an important risk marker for both preeclampsia and eclampsia.[2] The vascular effects of hyper homocysteinemia includes endothelial cell injury and thrombus formation. Elevated plasma homocysteine is an independent risk factor for peripheral vascular disease and coronary artery disease.[3]

Homocysteine is an essential amino acid required for growth of cells and tissues in the human body.[4,5] Homocysteine is eliminated from the body via conversion to cystathionine by a reaction catalysed by vitamin B6and to methionine catalysed by vitamin B12 and folic acid. Homocysteine is found in low concentrations in all tissues under normal conditions whereas it accumulates in tissues and plasma if these catalytic vitamins were depleted.

Levels of maternal serum homocysteine normally decrease with gestation, either due to a physiological response to the pregnancy or due to decrease in albumin, increase in estrogen, hemodilution from increased plasma volume and increased demand for methionine by both the mother and foetus.[6] Anotherpossible mechanism responsible for the reduction in homocysteine levels during pregnancy is its utilisation by the foetus. Folic acid supplementation resulted in higher serum red blood cell folate and lower homocysteine levels.

Hyperhomocysteinemia can result from mutations in methylene tetrahydro folate reductase (MTHFR) gene and cystathionine beta synthase (CBS) gene.[7] Hyperhomocysteinemia increases the risk of atherosclerosis through a mechanism involving oxidative damage. When added to plasma, homocysteine is readily oxidised to form homocystine, homocysteine mixed disulfides and homocysteine thiolactone leading to the formation of oxygen radicals and lipid peroxidation. Malondialdehyde, (MDA) a metabolite of lipid peroxides detectable in plasma is used as an indicator of lipidperoxidation.[8]

Homocysteine and hyperhomocysteinemia are relatively new concepts in obstetrics and gynaecology. Hyperhomocysteinemia in pregnant women has been associated with deep venous thrombosis, abruptio placenta, preeclampsia, neural tube defects, still birth and recurrent miscarriage.[9-15] By this study, we would like to know whether the earlier studies correlate with our population. Homocysteine may provide a missing link in the etiology of preeclampsia.

METHODS

The study was performed in Institute of maternal and child health, Medical College Kozhikode. Permission to conduct the study and ethics approval was obtained. The subjects were selected according to inclusion and exclusion criteria. Prior to registering, an informed written consent was taken. The present study was done by taking a statistical sample size of 30 subjects in each group.

Normotensive nonpregnant women (Control group) - 30

Normal pregnant women (Study group 1) - 30

Preeclampsia patients (Study group 2) - 30

All subjects were between 18-35years and of similar socio-economic class and dietary habits. Women with history of diabetes mellitus, chronic hypertension, renal or liver disease, history of thromboembolism, repeated miscarriage, abruption placenta, preterm labour and delivery, anaemia were excluded from the study. All of them abstained from smoking and alcoholism. Preeclampsia group includes antenatal patients having hypertension (BP ≥140/90mmHg) for the first time during pregnancy on more than two occasions and persistent proteinuria 30 mg / dl (>1±dip stick) in random urine samples after 20 weeks of pregnancy. All antenatal women were in the third trimester, i.e. between 28-40 weeks of gestation. A detailed history was taken from all the subjects.

Parameters assessed

Homocysteine assay was done using the Axis® Homocysteine Enzyme Immunoassay (EIA). Analysis of malondialdehyde was done using UV- Vis Spectrophotometer 118 (Systronics). Urine protein was analysed using dip stick method. Blood pressure measurement was done in all the subjects.

The present study was designed as a case control study and statistical analysis was done using Statistical Package for Social Sciences (SPSS) version 16. Analysis Of Variance (ANOVA) was used to test whether there is significant difference among two or more independent groups. The p value of <0.05 was taken as the level of significance. In order to find out whether there is a significant association or not between two variables, coefficient of correlation was calculated.

RESULTS

The mean serum homocysteine level of normal nonpregnant women (control group) was (13.31±5.81 µmol/ L) and that of normal pregnant women (study group 1) was (7.52±2.25 µmol / L) (Table 1) which is comparable to that reported by Kang et al.[16] The findings in this study were supported by the study of Walker et al which shows that homocysteine levels were significantly lower in pregnancy as compared to nonpregnant control.[6]

Table 1: Comparison of serum homocysteine levels in three groups.

	Homocysteine (µmol/L)			
Mean±SD	**Normal non- pregnant control group**	**Normal pregnant Group I**	**Preeclampsia Group II**	**p value**
	13.31±5.81	7.52±2.25	-	0.001 (significant)
	13.31±5.81	-	8.90±4.33	0.001 (significant)
	-	7.52±2.25	8.90±4.33	0.677 (not significant)

The homocysteine level of preeclamptic patients (8.90±4.33µmol/L) was found to be significantly lower than that of normal nonpregnant women (13.31±5.81µmol/L) (Table 1). While comparing the mean homocysteine level of preeclamptic patients (8.90±4.33µmol/L) with that of normal pregnant women (7.52±2.25µmol/L), the mean value was found to be higher but the difference was not statistically significant (Table 1). The preeclamptic patients in this study were on folic acid supplementation during their first trimester of gestation. This may be the reason for the minimally elevated homocysteine levels when compared to normal pregnant women. Another reason is that, majority of patients in this study belongs to mild preeclamptic category.

Table 2: Comparison of serum MDA levels in three groups.

	MDA (nmol/dL)			
	Normal non-pregnant control group	**Normal pregnant Group I**	**Preeclampsia Group II**	**p value**
Mean±SD	87.57±12.97	208.67±43.54	-	0.147 (not significant)
	87.57±12.97	-	352.13±52.69	0.001 (significant)
	-	208.67±43.54	352.13±52.69	0.001 (significant)

For better understanding the role of oxidative stress in the pathogenesis of preeclampsia, the lipid peroxidation product-malondialdehyde (MDA) levels were also assessed. The mean serum MDA level of normal pregnant women (208.67±43.54 nmol /dL) was found to be higher than that of normal nonpregnant women (87.57±12.97 nmol / dL), but the difference was not statistically significant (Table 2).

The mean serum MDA level of preeclampsia patients (352.13±52.69 nmol/dL) was significantly higher than normal nonpregnant (87.57±12.97nmol/dL) and normal pregnant women (208.67±43.54nmol /dL) (Table 2). These findings were consistent with the previous reports that oxidative stress plays an important role in the pathogenesis of preeclampsia. This may be due to the reactions between the diseased placenta and maternal dyslipidemia which occurs in preeclampsia. The present study also showed a positive correlation between serum homocysteine and MDA levels of preeclampsia patients.

DISCUSSION

The findings in this study suggest that homocysteine levels were directly correlated with the severity of preeclampsia. The results of this study were supported by Ingec M, et al which showed elevated homocysteine levels in severe preeclampsia and eclampsia, but not in mild preeclampsia.[17] The minimally affected endothelial dysfunction due to low levels of homocysteine may be a valid explanation for this finding.

Various hypotheses explaining the decrease in total homocysteine concentration during pregnancy have been proposed. Among these are hormonal influences on homocysteine metabolism, maternal dietary protein intake, hemodilution, decrease levels of albumin, as well as increased demand for methionine by both mother and fetus and folic acid supplementation.[18,19] The total homocysteine levels were found to be significantly lower in the luteal phase of the menstrual cycle than in follicular phase.[20] The decrease in total homocysteine that occurs in normal pregnancy may be attributable to a physiological phenomenon resembling an extension of the luteal phase of the menstrual cycle.

It has been suggested that deficiencies of folate, vitamin B12 and vitamin B6 or genetic defects in their metabolism may result in mildly elevated homocysteine concentration in blood and urine. But the fact that the homocysteine level as well as folate, vitamin B12 and vitamin B6 levels fell as pregnancy progresses might be explained by the strong effect of hemodilution on all these determinants.[21]

It has been suggested by Stugers-Theunissen et al that alterations in the methionine requirement might explain the reduction in total homocysteine concentration in pregnancy.[22] Also the homocysteine was incorporated into fetal metabolic cycle. Thus the fetal uptake of homocysteine during the mid to late pregnancy could explain the reduction. Folic acid supplementation was found to enhance the physiologic reduction in total homocysteine concentration during normal pregnancy.[23] This may be the reason for the minimally elevated homocysteine concentration in preeclampsia patients when compared to that of normal pregnant women as obtained in this study.

The physiologic role of oxidative stress during normal pregnancy is not fully understood. It may result from the maternal response to pregnancy when spiral arterioles were transformed into low resistance vessels around the invading trophoblast. But the oxidative stress which occurs in normal pregnancy will not do much harm to the mother.

This is due to the increase in antioxidants like vitamin E with pregnancy progression compared with preconceptional values. Also the elevation of vitamin E seems to correspond with the increase in products of membrane damage with advancing duration of gestation.[24]

The present study showed a positive correlation between serum homocysteine and MDA levels of preeclampsia patients. Homocysteine on auto oxidation forms homocysteine, homocysteine mixed thiolactone leading to the formation of oxygen radicals and lipid peroxidation.[8]

The placental trophoblast NADPH oxidase is the principal source of free radical synthesis. Also placenta is rich in polyunsaturated fatty acids which could serve as a source of lipid peroxides. Late pregnancy was associated with the formation of susceptible, oxidizable particles like triglycerides and high LDL score which were also attacked by the ROS, being more marked in preeclampsia than in normal pregnancy. The increased MDA levels in preeclampsia patients was known to be due to increased generation of reactive oxygen species and increased oxygen demand along with reduction in activation of enzymes like superoxide dismutase, glutathione peroxidase and decreased concentration of antioxidants like vitamin C, vitamin E. Uzen et al and Benian et al have reported oxidative stress and decreased antioxidant activity in preeclampsia.[25]

Thus the diminution of the antioxidant response to the oxygenation stimulus results in oxidative stress. This may lead to trophoblastic degeneration and possibly contribute to impairment of trophoblastic invasion and diminished remodeling of spiral arteries. A defective response to an antioxidant stimulus therefore is one of the earliest events in preeclampsia.

The findings in this study showed high levels of homocysteine and malondialdehyde concentrations in preeclampsia patients than in normal pregnant women. Also the homocysteine levels of normal pregnant women were found to be lower than normal non pregnant women. Thus the high concentrations of homocysteine in preeclampsia patients which increases the oxidant and decreases the antioxidant activities, might be the mechanism of endothelial injury and hence vasospasm. Deleterious effects of free radicals include initiation of lipid peroxidation, oxidation of biomolecules, cellular dysfunction and these will initiate maternal endothelial dysfunction and leukocyte activation, recognized features of preeclampsia. Malondialdehyde – the end product of lipid peroxidation reflects the oxidative damage of the biological system.

CONCLUSION

Hyperhomocysteinemia in pre-eclampsia, is associated with increased lipid peroxidation, as well as with blunted endothelial dependent vasorelaxation and an altered phenotype of endothelium from anticoagulant to procoagulant state. These homocysteine mediated vascular and a metabolic change predisposes the affected persons towards hypertension during pregnancy and future cardiovascular disease.

Hence supplementation of vitamins like folic acid, antioxidants or nitric oxide donors, decreases homocysteine levels and also decreases the increased oxidant activity and restores the endothelial function. Therefore, these women should receive adequate counseling to urge them to adopt healthier habits and lifestyles and to seek periodic checkups, in order to detect cardiovascular disease in its early stages, before irreparable damage or even death ensues.

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REFERENCES

1. Roberts J, Taylor R, Musci T, Rodgers G, Hubel C, McLaughlin M. Preeclampsia: An endothelialcell disorder. Am J Obstet Gynecol. 1989;161:1200-4.
2. Rajkovic A, Catalano PM, Manilow MR. Elevated homocysteine levels with preeclampsia. Obstet Gynecol. 1997;90:168-71.
3. Refsum H, Ueland PM, Nygard O, Vollset SE. Homocysteine and cardiovascular disease. Annu. Rev.Med. 1998;49:3-62.
4. Refsum H, Ueland PM, Nygard O, Brattstrom L, Vollset SE. Total homocysteine and cardiovascular disease. J Int Med. 1999;246:425-54.
5. Miner S, Evrovski J, Cole D. Clinical chemistry and molecular biology of homocysteine metabolism: an update. Clin Biochem. 1996;30:189-201.
6. Walker MC, Smith GN, Perkins SL, Kelly EJ, Garner PR. Changes in homocysteine levels during normal pregnancy. Amer J Obst and Gyn. 1999;180(3):660- 4.
7. Miner S, Evrovski J, Cole D. Clinical chemistry and molecular biology of homocysteine metabolism: an update. Clin Biochem. 1996;30:189-201.
8. Loscalzo J. The oxidant stress of hypehomo-cystenemia. J Clin Invest. 1996;98:5-7.
9. den Heijer M, Koster T, Blom HJ, Bos GM, Briet E, Reitsma PH, et al. Hyperhomocystenemia as a risk factor for deep vein thrombosis. N Engl J Med. 1996;334:759- 62.
10. Rajkovic A, Catalano PM, Manilow MR. Elevated homocysteine levels with preeclampsia. Obstet Gynecol. 1997;90:168- 71.
11. Wouters MG, Thomas CM, Boers GH, et al. Hyperhomocystenemia: a risk factor in women with unexplained recurrent early pregnancy loss. Fertil Steril. 1993;60:820-5.

12. Ray JG, Laskin CA. Folic acid and homocysteine metabolic defects and the risk of placental abruption, preeclampsia and spontaneous pregnancy loss. A systemic review. Placenta. 1999;20:519-29.
13. Dekker G, de Varies J, Doelitzsch P. Underlying disorders associated with severe early- onset preeclampsia. Am J Obstet Gynecol. 1995;173:1042-8.
14. Goddijin Wessel T, Wouters M, Molen E. Hyperhomocystenemia A risk factor for placental abruption or infarction. Eurt J Obstet Gynecol Reprod Biol. 1996;66:23-9.
15. Powers R, Evans R, Majors A. Plasma homocysteine concentration is increased in preeclampsia and associated with evidence of endothelial activation. Am J Obstet Gynecol. 1998;179:1605-11.
16. Kang SS, Wong PW, Norusis M. Homocysteinemia due to folate deficiency. Metabolism. 1987;36:458-62.
17. Ingec M, Borekci B, Kadanali S. Elevated plasma homocysteine concentrations in severe preeclampsia and eclampsia. Tohoku J. Exp. Med. 2005;206(3):225-31.
18. Steegers-Theunissen RPM, Wathen NC, Eskes TKAB, van Raaij-Selten B, Chard T. Maternal and fetal levels of methionine and homocysteinein early pregnancy. Br J Obstet Gynaecol. 1997;104:20-4.
19. Anderson A, Hultberg B, Brattstrom L, IsakSson A. Decreased serum homocysteine in pregnancy. Eur J Clin Chem. 1999;30:377-9.
20. Tallova J, Tomandl J, Bicikova M, Hill M. Changes of plasma total homocysteine levels during the menstrual cycle. Eur J Clin Invest. 1999;29:1041-4.
21. Kang SS, Wong PW, Zhou JM, Cook HY. Total homocysteine in plasma and amniotic fluid of pregnant women. Metabolism. 1986;35:889-91.
22. Steegers-Theunissen RPM, Wathen NC, Eskes TKAB, van Raaij-Selten B, Chard T. Maternal and fetal levels of methionine and homocysteine in early pregnancy. Br J Obstet Gynaecol. 1997;104:20-4.
23. Anderson A, Hultberg B, Brattstrom L, IsakSson A. Decreased serum homocysteine in pregnancy. Eur J Clin Chem. 1999;30:377-9.
24. Wang YP, Walsh SW, Guo JD. Maternal levels of prostacyclin, thromboxane, Vitamin E and lipid peroxides throughout normal pregnancy. Am J Obstet Gynecol. 1991;165:1690-4.
25. Uzen H, Benian A, Madazh R, Topcuoglu MA, Aydin S, Albayrak M. Circulating oxidized low density lipoprotein and paraoxanase activity preeclampsia. Gynecol Obstet Invest. 2005;60:195-200.

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