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EVALUATION OF OXIDATIVE STRESS IN PREGNANCY INDUCED HYPERTENSION

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ABSTRACT

This work was undertaken to investigate correlation between oxidative stress and initiation of pathogenesis of pregnancy induced hypertension (PIH). Fifty primigravidae in age group of 20-35 years and gestational age 28-42 weeks with PIH were taken as cases. Twenty healthy primigravidae with no medical and surgical complicatiions of pregnancy and with blood pressure ≤ 140/90 mm Hg served as controls. The cases were again subgrouped as severe preeclampsia (12 in number) and mild pre-eclampsia (38 in number). All of them were evaluated for serum malondialdehyde (MDA), Serum vitamin E and plasma vitamin C levels. The serum MDA levels were raised significantly in women with mild preeclampsia ($P<0.01$) and in women with severe preeclampsia ($P<0.01$) in comparison to normal primi gravida. The serum vitamin E levels were decreased in primi gravida with mild preeclampsia ($p<0.1$) and in primi with severe pre eclampsia ($P<0.1$) in comparison to normal primi gravida but the fall was not statistically significant. There was a significant fall ($P < 0.05$) in the vitamin C levels in primi with mild preeclampsia than in the normal primi. The vitamin C levels in severe preeclamptic patients were lower than the normal primi but the fall was not statistically significant ($P = 0.10$). The serum MDA and vitamin E showed a negative correlation in all the cases. The serum MDA and plasma vitamin C also showed a negative correlation in the control and study group. This observation suggests that in hypertensive disorders of pregnancy there is an imbalance between lipid peroxidation and antioxidant vitamin status because of oxidative stress. The decreased serum concentrations of the antioxidant vitamins supports the hypothesis that lipid peroxidation is an important causative factor in the pathogenesis of preeclampsia. The rise in antioxidants is probably to compensate the increased peroxide load in severe preeclampsia.

KEY WORDS

Lipid peroxides, vitamin E, ascorbic acid

INTRODUCTION

Hypertensive disorders are the most common medical complications of pregnancy, with a reported incidence ranging between five to ten per cent (1). The incidence varies among different hospitals, regions and countries. In India the incidence of preeclampsia is reported to be 8-10 per cent of the pregnancies (2). Hypertension in pregnancy strikes mostly the primigravidae after twentieth week of gestation and frequent occurences are seen near term. It contributes significantly to the cause of maternal and perinatal mortality and morbidity (3). Uncontrolled lipid peroxidation is a key contributing factor to pathophysiologic condition of preeclampsia (4, 5, 6, 7). It has also been hypothesized that reduction in the antioxidant activity may enhance endothelial cell oxidative damage but studies of various systems have produced conflicting results (6, 7, 8, 9, 10, 11, 12, 22, 28). It has been suggested that uncontrolled lipid peroxidation may play a role in the etiology of the PIH.

For the aforesaid reason we took up this work in the Department of Biochemistry, S.C.B. Medical College, Cuttack in collaboration with the Department of Obstetrics and Gynaecology to find out the relationship of MDA, vitamin E and vitamin C in our series of patients and to study if there is any correlation between the serum antioxidant levels to that of lipid peroxidation product.

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MATERIALS AND METHODS

Pregnant women attending Antenatal OPD and Labour

room of the Department of Obstetrics and Gynaecology, S.C.B. Medical College, Hospital, Cuttack, were selected for the study. Primigravidae in the age group of 20-35 years with blood pressure > 140/90 mm Hg with oedema and proteinuria and within 28-42 weeks of gestation constituted the study group. The patients in the study group were further classified as :

(a) Primi with mild preeclampsia : with B.P. > 140/90-150/109 mm Hg and urinary protein > 0.3 gm/day.

(b) Primi with severe preeclampsia : with B.P. > 160/100 mm Hg and urinary protein > 3 gm/ day.

Age matched primigravidae with blood pressure $\leq$ 140/90 mm Hg without oedema or proteinuria and within 28-42 weeks of gestation constituted the control group. All the women were of low socio-economic group.

About 7 ml of fasting venous blood sample was collected and the following parameters were estimated in the controls and cases.

1. Serum malondialdehyde level (K Satoh, 1978), (13).
2. Serum vitamin E level (Baker and Frank, 1968), (14).
3. Plasma vitamin C level (Harris and Ray, 1935), (15).
4. Urinary protein estimation (by trubidimetric method using sulphosalicylic acid, Harold varley). (16). This test was carried out to differentiate the patient with mild preeclampsia from the patient with severe pre-eclampsia.

Statistical analysis

Student's t-test was employed for the statistical analyses of data to compare each group. The data was expressed as mean ± standard error. p-value less than 0.05 was taken as the significant value pearson's correlation coefficients were used to compare the correlations.

RESULTS

There was a rise in serum MDA level in primi gravidae with mild preeclampsia (4.9 ± 2.02 nmol/ml) and primi within severe preeclampsia (5.87 ± 1.64 n mol/ml) in comparison to normal primi (2.48 ± 1.47 n mol/ml), the rise being highly significant (P < 0.01) (Table 2). Although an increase in serum MDA level was seen in primi with severe preeclampsia (5.87 ± 1.64 n mol/ml) as compared to primi with mild preeclampsia (4.90 ± 2.02 n mol/ml) the rise was not statistically significant (p < 0.01) (Table 2).

In our study the serum vitamin E level was found to be decreased in primi with mild preeclampsia (7.11 ± 2.57 mol/l) as compared to normal primigravidae (8.35 ± 3.54 mol/l) but the fall was not statistically significant (P < 0.10) (Table 2).

The serum vitamin E level was decreased in primi with severe preeclampsia (7.73 ± 3.29 mol/l) when compared to normal primigravidae (8.35 ± 3.54 mol/l), but the fall was not statistically significant (P < 0.10), as shown in Table 2.

When we compared the serum vitamin E levels in patients with mild preeclampsia (7.11 ± 2.57 mol/l) with severe preeclamptic patients (7.73 ± 3.29 mol/l) an increase was observed in the later group, but the increase was also not statistically significant (p > 0.10).

Table 1. Clinical Parameters in control and study groups.

	Normal Primi Gravida (n = 20)	Primi with mild Preeclampsia (n = 38)	Primi with Severe Preeclampsia (n = 12)
Age in years (mean)	24.55	25.98	28.58
Mean gestational age in weeks	36.8	32.65	30.16
Mean Systolic blood pressure in mm Hg	119.4	146.31	168.33
Mean diastolic blood pressure in mm Hg	79.5	97.63	113.0
Proteinuria (mean) in gm / day	Nil	0.95	3.6
Oedema	Nil	Nil to + in all cases	++ in all cases

Table 2.

Study group	Serum M.D.A. in nmol/ml	Significance (p)	Serum vitamin E in mol/l	Significance (p)	Plasma vitamin C in mg/l	Significance (p)
Normal primi Gravida (n = 20)	2.48 ± 1.47		8.35 ± 3.54		8.55 ± 3.74	
Primi with mild preeclampsia (n = 38)	4.90 ± 2.02	$p < 0.01^{\#}$	7.11 ± 2.57	$p < 0.10^{\#}$	6.68 ± 2.42	$p < 0.05^{\#}$
Primi with servere preeclampsia (n = 12)	5.87 ± 1.64	$p < 0.10^{@}$ p < 0.01 ®	7.73 ± 3.29	$p > 0.10^{@}$ $p < 0.10^{\$}$	6.93 ± 2.00	$p > 0.10^{@}$ $p = 0.10^{\$}$

Data are presented as mean ± S.D.
Comparison between normal primi and primi with mild preeclampsia
@ Comparison between primi with mild preeclampsia and primi with severe preeclampsia.
$ Comparison between normal primi and primi with severe preeclampsia.

Table 3.

Correlation coefficent (r)	Normal Primi Gravida (n = 20)	Primi with mild Preeclampsia (n = 38)	Primi with Severe Preeclampsia (n = 12)
Correlation between serum MDA with Serum vitamin E	-0.513 (p < 0.05)*	-0.332 (p < 0.05)*	-0.329 (p > 0.10)
Correlation between serum MDA with Serum vitamin C	-0.163 (p < 0.10)	-0.507 (p < 0.01)	-0.290 (p > 0.10)

* Significant (negative) correlation.

The plasma vitamin C values as obtained in our study were (8.55 ± 3.74 mg/l) in normal primigravidae (6.68 ± 2.45 mg/l) in primi with mild preeclampsia and (6.93 ± 2.00 mg/l) in primi with severe preeclampsia (Table 2). There was a significant fall (p < 0.05) in vitamin C level in primi with mild preeclampsia in comparison to normal primigravidae. The vitamin C levels in the patients with severe preeclampsia were found to be more than the patients with mild preeclampsia but the difference was not statistically significant (P > 0.10) (Table 2). However, the vitamin C levels in them were found to be lower than the normal primigravidae but the fall was not statistically significant (p = 0.10).

DISCUSSION

We have observed a significant rise (P < 0.01) in serum MDA levels in both the sub group of cases in comparison to the control group. An increase in lipid peroxidation in preeclamptic patients as compared with normotensive pregnant women was also observed by other workers (10, 17, 18). The elevated plasma concentration of free radical oxidation product precede the development of preeclampsia. The lipid peroxides and free radicals may be important in pathogenesis of preeclampsia (7, 19). But some studies have reported that there is no evidence of increased lipid peroxidation in PIH (20).

Our study agrees with other workers (10, 11, 12, 21, 22) though the fall in vitamin E level in both the subgroups of cases as compared to the control was not significant. This suggests that there is an imbalance between the rise in lipid peroxidation and fall in the defensive antioxidant mechanism. Vitmain E is a free radical scavenger and exerts its antioxidant

activity in lipid phase. However, vitamin E is consumed in exerting its action. Hence abnormal rise in lipid peroxides in preeclampsia could increase consumption of antioxidants resulting in decreased vitamin E levels. Another possibility is decreased absorption of vitamin E from the gut as a result of vasoconstriction in preeclampsia.

The serum vitamin E levels in patients with severe preeclampsia were found to be more in comparison to patients with mild preeclampsia though the rise was not significant ($P > 0.10$). Vitamin E is known to be mobilised from the tissues to the sites of oxidative stress (23). This could indicate increasing oxidative stress with continuation of pregnancy in severe preeclampsia (24) which evokes the antioxidative response. The increase in vitamin E level in severe preeclampsia occur to counter the raised lipid peroxidation with the severity of the disease process. Our study agrees with other authors (6, 25, 28) who have shown an increase in serum vitamin E levels in pregnancy induced hypertension, in mild preeclampsia with HELLP syndrome and in severe preeclampsia.

There was a significant fall ($p < 0.05$) in vitamin C levels in primi with mild preeclampsia in comparison to normal primigrvidae but the fall in the levels of plasma vitamin C levels in patients with severe preeclampsia was not significant ($P = 0.10$) when compared with normal primi. Our study agrees well with the study of other workers (9, 10, 22, 28). Reduced ascorbic acid, as a water soluble antioxidant was reported to function as the first-line anti-oxidant defence against free oxygen radicals present primarily in plasma (26). In contrast, alpha tocopherol is a lipid soluble antioxidant that has the capacity to quench free oxygen radicals present primarily in lipid cell membrane (27). When the capacity of ascorbic acid is exceeded, free radicals can then diffuse to cell membrane initiating lipid peroxidation (29), the propagation of which can be inhibited by alpha tocopherol.

We had noted a marked increase in "Oxidative Stress" in preeclamptic patients as compared to normal pregnant women. This is evident from our observations regarding the ratios of mean values of MDA to vitamin E in normal primi (0.297), primi with mild preeclampsia (0.688), and primi with severe preeclampsia (0.795) and the ratios of mean values of MDA to vitamin C in normal primi gravidae (0.290), primi with mild preeclampsia (0.733), primi with severe preeclampsia (0.847). Our study correlates well with other workers (9).

The increase in oxidative stress was further supported by the observation of a negative correlation between lipid peroxidation products (MDA) with antioxidants vitamin E and vitamin C.

From the above observations we conclude that with increase in severity of the disease the oxidative stress increases. Hence there is a definite correlation between oxidative stress and initiation of pathogenesis of PIH.

Reference

1. Sibai, B.M. (1992). Hypertension in pregnancy. Obstet. Gynecol. Clin. North Am. 19, 615.
2. Krishna Menon, M.K. and Palaniappan, B. (1994). Hypertensive disorders of pregnancy. In Mudaliar Menon (ed.). Clinical Obstetrics. 9th edn. Orient Longman, Madras, 133-154.
3. National High Blood Pressure Education Programme Working Group. 1990.
4. Hubel, C.A., Roberts, J.M., Taylor, R.N., *et al*. (1989). Lipid peroxidation in pregnancy : New perspectives on preeclampsia. Am. J. Obstet. Gynecol. 161, 1025-1034.
5. Wang, Y., Walsh, S.W. and Kay, H.H. (1992). Placental lipid peroxides and thromboxane are increased and prostacyclin is decreased in women with preeclampsia. Am. J. Obstet. Gynecol. 167, 946-949.
6. Uotila, J.T., Tuimala, R.J., Aarino, T.M., *et al*. (1993). Findings on lipid peroxidation and anti oxidant function in hypertensive complications of pregnancy. Br. J. Obstet. Gynaecol. 100 (3), 270-276.
7. Wu, J.J. (1996). Lipid peroxidation in preeclamptic and eclamptic pregnancies. Eur. J. Obstet. Gynecol. Reprod. Biol. 64 (1), 51-54.
8. Davidge, S.T., Hubel, C.A., Brayden, R.D., *et al*. (1992). Sera antioxidant activity in uncomplicated and preeclamptic pregnancies. Obstet. Gynecol. 71, 897-901.
9. Wisdom, S.J., Wilson, R., Mckillop, J.H., *et al*. (1991). Antioxidant systems in normal pregnancy and pregnancy induced hypertension. Am. J. Obstet. Gynecol. 165, 1701-1704.
10. Wang, Y., Walsh, S.W., Gu, J., *et al.* (1991). The imbalance between thromboxane and prostacyclin in preeclampsia is associated with an imbalance between lipid peroxides and vitamin E in maternal blood. Am. J. Obstet. Gynecol. 165, 1965-1700.
11. Mikhail, M.S., Anyaegbunam, A., Garfinkel, D., Palan, P.R., Basu, J. and Romney, S.L. (1994). Preeclampsia and antioxidant nutrients, decreased plasma levels of reduced ascorbic acid, alpha-tocopherol and Beta-Carotene in women with preeclampsia. Am. J. Obstet. and Gynecol. 171 (1), 150-157.

12. Iioka, H. (1994). Changes in blood le;vel of lipid peroxide and Vitamin E during pregnancy clinical significance and relation to the pathogenesis of EPH gestosis. Gynecol. Obstet. Invest. 38, 173-176.

13. Satoh, K. (1978). Clinica. Chemica. Acta. 37-43.

14. Baker, H., Frank, D. and Winley, N.C. (1968). Clinical vitaminology, 772.

15. Harris and Ray (1935). In Vitamins In Practical Clinical Biochemistry. Harold Varley Alan H. Gowenlok, Maurice Bell (5th edn.). William Heinmann Medical Books Ltd., London.

16. Harold Varley. Estimation of urinary proteins by Turbidimetric method using sulphosalicylic Acid. In Practical Clinical Biochemistry (5th edn.). Vol. 1, p. 606.

17. Wickens, D., Wilkins, M.H., Lunec, J., Ball, G. and Dormandy, T.L. (1981). Free radical oxidation (Peroxidation) products in plasma in normal and abnormal pregnancy. Ann. Clin. Biochem. 18, 158-162.

18. Tsukatani, E. (1983). Etiology of EPH - gestosis from the view point of dynamics of vasoconstrictive prostanoid, lipid peroxides and vitamin E. Acta. Obstet. Gynecol. Jpn. 35, 713-720.

19. Walsh, S.W. (1994). Lipid peroxidation in pregnancy, Hypertension in pregnancy. Am. J. Obstet. Gynecol. 13, 1;

20. Regan, C.L., Levine, R.J., Baird, D.D., Ewell, M.G., Martz, K.L., Sibai, B.M., Rokach, J., Lawson, J.A. and Fitazgerald, G.A. (2001). No evidence for lipid peroxidation in severe preeclampsia. Am. J. Obstet. Gynecol. 185 (3), 572-578.

21. Jain, S.K. and Wise, S.R. (1995). Relationship between elevated lipid peroxides, vitramin E deficiency and hypertension in preeclampsia. Molecular and Cellular Biochemistry 151 (1), 33-38.

22. Kwasniewska, A., Tukendorf, A. and Semczuk, M. (1998). Serum antioxidant concentrations in pregnancy induced hypertension. Med. Sci. Monit. 4 (3), 44.

23. Brown, K.M., Morrice, P.C. and Duthie, G.G. (1994). Vitamin E supplementation supresses indexes of lipid peroxidation and platelet counts in blood of smokers and non-smokers but plasma lipoprotein concentrations remain unchanged. Am. J. Clin. Nutr. 60, 383-387.

24. Gulmezoglu, A.M., Hofmeyr, G.J., and Mathys, M.J. Oosthuisen (1997). Antioxidants in the treatment of severe preeclampsia an explanatory randomized controlled triat. British Journal of Obstet. and Gynacol. 104, 689-696.

25. Eyal, Schiff, Steven, A. Friedman, Meir Stampfer, *et al*. (1996). Dietary consumption and plasma concentrations of vitamin E in pregnancies complicated by preeclampsia. Am. J. Obstet. Gynecol. 175 (4) : 1024-1028.

26. Wefers, M. and Sies, H. (1988). The protection by ascorbate and glutathione against microsomal lipid peroxidation is dependent on vitamin E. Exp. J. Biochem. 174 : 353-357.

27. Niki, E., Yamamoto, Y., Komuro, E. and Sato, K. (1991). Membrane damage due to lipid oxidation. Am. J. Clin. Nutr. 53, 201-205.

28. Rao, G.M., Sumita, P. and Roshrim, M.N. Ashtagimatt (2005). Plasma antioxidant vitamins and lipid peroxidation products in pregnancy induced hypertension. India J. of Clin. Biochemistry 20 (1), 198-200.

29. Frei, B., England, L. and Ames, B.N. (1989). Ascorbate is an outstanding antioxidant in human blood plasma. Proc. Natl. Acad. Sci. USA 86, 6377-6381.