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STATUS OF LIPID PEROXIDATION, GLUTATHIONE, ASCORBIC ACID, VITAMIN E AND ANTIOXIDANT ENZYMES IN PATIENTS WITH PREGNANCY – INDUCED HYPERTENSION

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Abstract : The exact pro-oxidant and antioxidant status in pregnancy - induced hypertension patients is still not clear. To add a new insight to the question, changes in the erythrocyte lipid peroxidation products (malondialdehyde; MDA), levels of glutathione (GSH), ascorbic acid and plasma vitamin E (non enzymatic antioxidant parameters) and activities of antioxidant enzymes superoxide dismutase (SOD), glutathione peroxidase (GP_X), catalase in erythrocytes were studied in thirty five patients with pregnancy – induced hypertension and thirty five healthy pregnant normotensive cases. It was observed that there was a significant increase in erythrocyte MDA levels, activities of SOD, GP_X and a significant decrease in erythrocyte GSH, ascorbic acid, plasma vitamin E levels and catalase activity in patients with pregnancy - induced hypertension when compared to controls. The results of our study have shown higher oxygen free radical production, evidenced by increased levels of MDA and decreased levels of GSH, ascorbic acid, vitamin E and Catalase activity supports the oxidative stress in pregnancy - induced hypertension. The increased activities of antioxidant enzymes may be a compensatory regulation in response to increased oxidative stress. The decreased concentrations of glutathione and antioxidant vitamin status supports the hypothesis that lipid peroxidation is an important causative factor in the pathogenesis of preeclampsia.

Key words : malondialdehyde (MDA) glutathione (GSH) ascorbic acid superoxide dismutase (SOD) catalase vitamin E glutathione peroxidase (GP_X) pregnancy-induced hypertension

INTRODUCTION

Hypertension is one of the common problems in pregnancy and causes morbidity and mortality to both mother and child. Pregnancy - induced hypertension (PIH) is a syndrome of hypertension in pregnancy with or with out proteinuria and oedema. If associated with proteinuria then the condition is known as preeclampsia, which may occur as early as 20 weeks of pregnancy. Hypertensive disorders are the most common medical complications of pregnancy with a reported incidence ranging between 5–10% (1). Preeclampsia is a common problem during pregnancy, affecting upto one in seven pregnant women around the world (2). In India the national incidence of PIH is

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15.2% and the incidence of preeclampsia is reported to be 8–10% of the pregnancies (3). PIH is associated with endothelial dysfunction (4) and could be caused by oxidative stress (5). The unsaturated lipids and thiol containing proteins of the cell membranes are susceptible to free radical attack. There are reports about increased free radical activity in PIH (6). Lipid peroxidation mediated by free radicals is considered to be the major mechanism of cell membrane destruction and cell damage and is a key contributing factor to pathophysiologic condition of preeclampsia (7). Free radicals are formed in both physiological and pathological conditions in mammalian tissues (8). The uncontrolled production of free radicals is considered as an important factor in the tissue damage induced by several conditions (9, 10). Alteration in the oxidant – antioxidant profile is known to occur in PIH (11). Moreover the body's defense mechanisms would play an important role in the form of antioxidants and try to minimize the damage, adapting to the above stressful situation. Antioxidants are compounds that dispose, scavenge, and suppress the formation of free radicals, or oppose their actions (12).

In the present study, the following parameters were assessed in erythrocytes and plasma to elucidate the oxidant-antioxidant status in patients with pregnancy-induced hypertension. Erythrocyte malondialdehyde (MDA) levels were measured as an index of extent of lipid peroxidation. Erythrocyte glutathione (GSH), ascorbic acid and plasma vitamin E served as non-enzymatic antioxidant parameters. The antioxidant enzymes superoxide dismutase (SOD), catalase, glutathione peroxidase (GP_X) in erythrocytes were also estimated.

METHODS

Thirty five pregnant women attending OPD and labour room of department of obstetrics and gynaecology of Dr. Pinnamaneni Siddhartha Institute of Medical Sciences and Research Foundation General Hospital, Chinoutpally, A.P. India were chosen for the study. An equal number of age matched healthy normotensive pregnant women were also investigated. The diagnosis of PIH was done as per the norms of American college of obstetrics and gynaecologists (13) that is systolic blood pressure ≥ 140 mmHg, diastolic blood pressure ≥ 90 mmHg or increase of ≥ 30 mmHg in systolic blood pressure or increase of ≥ 15 mmHg in diastolic blood pressure over the pre-pregnancy. PIH was diagnosed when any of these criteria were present at least on two occasions separated by an interval of 6 hours. Urinary protein estimation was also performed to check whether there is associated proteinuria or not. Clearance was obtained from the ethical committee of the institute before beginning of the work. Demographic details of PIH patients and control subjects were presented below.

Control group : Age matched thirty-five healthy pregnant normotensive women as controls [age (years): 25.4±0.6, gestational age (weeks) : 38.16±0.32, systolic B P (mm Hg) : 118±1.12, diastolic B P (mm Hg) : 78±1.26 with no proteinuria or oedema].

PIH group : Thirty-five women with pregnancy-induced hypertension [age (years) : 28.5±0.94, gestational age (weeks) : 36.02±0.18, systolic B P (mm Hg) : 164±1.82, diastolic B P (mm Hg) : 115±1.88, proteinuria (g/day) : 2.8±0.82, oedema was present in all cases).

The heparinised maternal venous blood samples were collected from these subjects and were used for the analysis. Plasma was separated by centrifugation at 1,000 g for 15 minutes. Separated plasma was used for the estimation of vitamin E. The buffy coat was removed and the packed cells were washed three times with physiological saline. The erythrocyte suspension was prepared by the method of Dodge et al., (14) modified by Quist (15). The packed cells were used for the estimation of GSH, ascorbic acid, MDA, SOD, catalase, GP_X. Erythrocyte GSH was estimated by the method of Beutler et al (16) using di thio bis nitro benzoic acid (DTNB). Ascorbic acid levels were estimated by the method of Tietz (17). Plasma vitamin E levels were estimated by the method of Baker et al (18). MDA was determined as the measure of thio barbituric acid reactive substances (TBARS) (19). SOD (EC 1.15.1.1) activity was determined in the hemolysate by the method of Misra and Fridovich (20) based on the inhibition of auto oxidation of epinephrine to adenochrome at pH 10.2. Catalase (EC 1.11.1.6) activity was measured by the method of Beers and Sizer (21). The activity of glutathione peroxidase (GP_X, EC 1.11.1.9) was measured as described by Paglia and Valentine (22) in erythrocytes and urinary protein estimation was done by turbidometric method using sulphosalicylic acid (23). All reagents used were of analytical reagent grade. DTNB and thio barbituric acid were obtained from Sigma chemicals, St.Louis, MO.

Statistical analysis

Statistical analysis between control group and PIH group was performed by the Student's t test for unpaired observations. The data were expressed as mean±SD. $P<0.05$ was considered as significant.

RESULTS

The mean±SD of erythrocyte GSH, ascorbic acid, MDA, SOD, catalase, GP_x, plasma vitamin E were indicated in the Table I. There was a statistically significant increase in the erythrocyte MDA levels in patients with PIH as compared to controls. The activities of erythrocyte antioxidant enzymes SOD and GP_X were significantly increased in PIH patients as compared to controls.

The levels of erythrocyte GSH, ascorbic acid, plasma vitamin E and catalase activity were significantly decreased in PIH patients as compared to controls.

TABLE I: The mean±SD values of malondialdehyde (MDA), glutathione, ascorbic acid, vitamin E, superoxide dismutase (SOD), catalase and glutathione peroxidase (GP_X) in controls and patients with pregnancy - induced hypertension (PIH).

Parameters	*Control group n=35*	*PIH group n=35*
Glutathione (mg/g of Hb)	18.89±2.48	10.39±1.26**
Ascorbic Acid (mg/dl)	6.06±0.31	5.92±0.16**
Vitamin E(umoles/L)	7.47±0.33	6.95±0.22***
MDA (nmoles/g of Hb)	8.67±0.22	9.92±0.28***
SOD (EU/g of Hb)	3756±469.8	4611±402.2***
Catalase (nmole H_2O_2 decomposed/mg protein/1 min)	5.33±0.31	4.49±0.15*
GP_X (U/g of Hb)	64.75±2.53	66.38±1.86***

*P<0.05; **P<0.01; ***P<0.001 as compared to controls.

DISCUSSION

In the present study the lipid peroxidation product i.e. malondi aldehyde (MDA) levels have been increased significantly in erythrocytes of the patients with pregnancy-induced hypertension. Rise

in MDA could be due to increased generation of reactive oxygen species (ROS) due to the excessive oxidative damage generated in these patients. These oxygen species in turn can oxidize many other important biomolecules including membrane lipids. The lipid peroxides and free radicals may be important in pathogenesis of preeclampsia. Sharma et al found raised oxidative stress and low antioxidant status in PIH (24). In contrast to our observation some studies have reported that there is no evidence of increased lipid peroxidation in PIH (25).

We observed a significant decrease in the levels of erythrocyte glutathione (GSH), ascorbic acid and plasma vitamin E (non enzymatic antioxidant defense system) in patients with PIH when compared to controls. The decrease in the levels of these non-enzymatic antioxidant parameters may be due to the increased turnover, for preventing oxidative damage in these patients suggesting an increased defense against oxidant damage in PIH.

In our study the erythrocyte antioxidant enzyme i.e. superoxide dismutase (SOD) and glutathione peroxidase (GP_x) activities have been increased significantly in patients with PIH. SOD is the important antioxidant enzyme having an antitoxic effect against super oxide anion. The over expression of SOD might be an adaptive response and it results in increased dismutation of superoxide to hydrogen peroxide. GP_X, an oxidative stress inducible enzyme plays a significant role in the peroxyl scavenging mechanism and in maintaining functional integration of the cell membranes (26). The rise in the activity of GP_X could be due to its induction to counter the effect of increased oxidative stress.

In the present study, we have observed a significant decrease in the activity of catalase in patients with PIH as compared to controls. Catalase is the enzyme, which protects the cells from the accumulation of hydrogen peroxide by dismutating it to form water and oxygen or by using it as an oxidant in which it works as a peroxidase (27).

In conclusion, results of our study have shown higher oxygen free radical production and decreased catalase activity, supports the higher oxidative stress hypothesis in pregnancy-induced hypertension. The increased activities of antioxidant enzymes may be a compensatory regulation in response to increased oxidative stress. Lipid peroxides could be a part of the cytotoxic mechanisms leading to the endothelial injury. The decreased concentrations of the glutathione and antioxidant vitamin status supports the hypothesis that lipid peroxidation is an important causative factor in the pathogenesis of preeclampsia. Our findings support the studies considering lipid peroxidation as an important factor in the pathogenesis of PIH (28). Therefore, the treatment with antioxidants in the initial stages of the disease may be useful as secondary therapy to prevent the oxidative damage.

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Lipid peroxidation and antioxidant status in maternal and cord blood. Gynecol. Obstet.Â Status of lipid peroxidation, glutathione, ascorbic acid, vitamin E and antioxidant enzymes in patients with pregnancy-induced hypertension. Indian J. Physiol. Pharmacol., 51: 284-288. eISSN: 09748369, www.biolmedonline.com. Lipid peroxidation, glutathione, ascorbic acid, vitamin E, antioxidant enzyme and serum homocysteine status in patients. with polycystic ovary syndrome. 1Surapaneni Krishna Mohan*, 2Vishnu Priya V 1Department of Biochemistry, Saveetha Medical College & Hospital, Saveetha University, Saveetha NagarÂ Abstract The exact pro-oxidant and antioxidant status in patients with polycystic ovary syndrome is still not clear. Many studies suggest that polycystic ovary syndrome may increase risk for several conditions like type 2 diabetes, dyslipidemia, endometrial cancer and hypertension.