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CORRELATION OF HOMOCYSTEINE AND OXIDATIVE STRESS IN PATIENTS WITH PRE-ECLAMPSIA.

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ABSTRACT

Preeclampsia is a frequent and potentially dangerous complication of pregnancy. A case control study was carried out to assess the levels of homocysteine, lipid peroxidation marker malondialdehyde (MDA) and antioxidant enzymes glutathione peroxidase (GPx) and superoxide dismutase (SOD) in patients with preeclampsia. The levels of malondialdehyde and homocysteine was significantly increased in patients with preeclampsia ($p<0.001$). The levels of GPx and SOD were significantly increased in patients with preeclampsia ($p<0.001$).Levels of homocysteine correlated positively with the levels of MDA and negatively with GPx and SOD. Oxidative stress may thus be a possible link in between homocysteine and preeclampsia.

KEY WORDS: Preeclampsia, homocysteine, malondialdehyde, glutathione peroxidase, superoxide dismutase

INTRODUCTION

Pre-eclampsia is a pregnancy-specific condition characterized by hypertension and proteinuria that remits after delivery. Preeclampsia affects between 0.4% and 2.8% of all pregnancies in developed countries and many more in developing countries, leading to as many as 8, 370, 000 cases worldwide per year. This common disorder, which is more prevalent in first pregnancies, is associated with the highest maternal and fetal morbidity and mortality of all pregnancy complications, with >90% of the most serious outcomes occurring in developing countries [1]

The cause of pre-eclampsia remains largely unknown, but poor placentation is an important predisposing factor. This proposed role of the placenta in the pathology of preeclampsia is also strongly supported by the rapid resolution of symptoms after delivery.

Homocysteine, a thiol-containing amino acid produced by the intracellular demethylation of methionine in the methylation processes. is receiving a lot of attention these days as a new risk factor for a variety of disease. [2]One mechanism by which increased homocysteine has been imposed to influence its pathological effects is by promoting oxidative stress [3,4]

A current theory holds that oxidative stress, ie, an imbalance between maternal prooxidants and antioxidants, is a component of preeclampsia. [5]It is uncertain whether such an imbalance occurs before clinical recognition of the syndrome or whether it is related to diet.

Enhanced placental superoxide generation lead to the generation of free radicals. [6] Deleterious effects of free radicals include initiation of lipid peroxidation, oxidative damage of biomolecules, and cellular dysfunction, and it is proposed that these may initiate maternal vascular endothelial dysfunction and leukocyte activation.

Basic research during the last decade has led to an increased association of oxidative stress with homocysteine in a variety of diseases. [7] To our knowledge little is known regarding the association of oxidative stress and homocysteine in patients with preeclampsia. Hence the present study was carried out to correlate the levels of oxidative stress and homocysteine in preeclamptic patients.

MATERIAL AND METHOD

Females in the third trimester of their pregnancy attending the antenatal clinic or admitted to the maternity ward of NKP Salve Institute of Medical Sciences, Nagpur were enrolled in this prospective study. The ethical institutional committee approved the study and all participants gave written informed consent. 50 of preeclampsia (i.e., high blood pressure [BP] and proteinuria) and 50 of normal pregnancy were enrolled for the study. 50 normal controls were age and sex matched with the normal pregnant and preeclamptic patients. Pre-eclampsia was defined as systolic and diastolic BP greater than 140 mm Hg and 90 mm Hg, respectively, with significant proteinuria (N300 mg per 24 h); mild pre-eclampsia was defined as diastolic BP less than 110 mm Hg,with significant proteinuria; and severe preeclampsia as diastolic

BP greater than 110mm Hg, or massive proteinuria (N2 g/24 h), or serum creatinine level greater than 1.2 mg/dL, or when other signs and symptoms of severe pre-eclampsia such as persistent headache, visual disturbances, persistent epigastric pain, and/or thrombocytopenia were present [8]. Detailed patient history was taken and a physical examination performed. Blood pressure was measured in the left arm with a syphygomanometer. Urinalysis was done for proteinuria. Patients with other associated disorders and anemic patients were excluded from the study. A total of 10 mL of venous blood was taken from all women. All blood samples were drawn into tubes free of endotoxins. The tubes were centrifuged for 10 min at 4000 rpm, plasma was separated, and packed erythrocytes were washed 3 times. Serum malondialdehyde (MDA), a marker of the oxidant status was determined by method of randox laboratory. This method was based on the fact that lipid peroxide condense with 1 methyl-2 phenyl indole under acidic conditions resulting in the formation of a red chromophore. To determine specifically lipid peroxide in plasma, proteins are precipitated to remove water-soluble MPI reactive substance. The level of lipid peroxide is expressed in terms of malondialdehyde, which is unstable. Tetramethoxypropane, which is converted quantitatively to MDA in the reaction procedure is used as standard.

Erythrocytic glutathione peroxidase (GPx) was estimated by enzymatic kit method, the principle being that GPx catalyses the oxidation of glutathione by cumene hydroperoxide. In the presence of glutathione reductase and NADPH the oxidized glutathione is immediately converted to reduced form with a concomitant oxidation of NADPH to NADP. The decrease in absorbance at 340 nm is measured.

Superoxide dismutase (SOD) was measured by enzymatic kit method. The principle employs xanthine and xanthine oxidase to generate superoxide radicals which react with 2-(4-iodophenyl)-3-(4- nitrophenol)-5-phenyltetrazolium chloride to form a red formazan dye.
For the measurement of homocysteine, all the specimens were transported to the laboratory within 30 minutes of collection. Thereafter, specimens were centrifuged for 5-7 minutes at 3000 rpm. Then clear serum was transferred in a plastic vial and stored in refrigerator until analysis. Homocysteine was measured by Microplate enzyme immunoassay kit method of Biorad Laboratories.

Statistical significance of difference was estimated using students `t` test and correlation between variables was studied by using Pearson`s correlation coefficient test.

RESULTS

Our results depict an increase in the values of MDA and homocysteine in patients with preeclampsia ($p<0.001$) when compared with normal healthy controls and normal pregnant females. However, there is a decrease in the levels of antioxidant enzyme SOD and GPx in preeclamptic patients when compared to normal healthy controls and normal pregnant females($p<0.001$). Corelation analysis reveals that MDA and homocysteine have a positive correlation (0.9; $p<0.001$), where as they have a negative correlation with antioxidant enzyme SOD(-0.486, -0.55; $p<0.001$) and GPx(-0.61, -0.52; $p<0.001$) respectively in preeclamptic patients

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TABLE I-Oxidant and antioxidant status in healthy controls and patients of preeclampsia (mean ± SD)

	Control (n= 50)	Normal pregnancy(n=50)	Preeclamptic patients (n= 50)
Serum MDA (nmol/ml)	0.96 ± 0.12	1.77 ± 0.23	2.24 ± 0.43*
Erythrocytic SOD (U/g m Hb)	6.14 ± 0.98	5.58 ± 0.31	4.05 ± 0.6 *
Erythrocytic GPx (U/gm Hb)	16.36 ± 0.54	15.23 ± 1.3	12.97 ± 0.57*
Serum Homocysteine (µmol/L)	17.88 ± 6.71	12.56 ± 5.47	55 ± 19.0*

*p<0.001(vs the pregnant patients)

TABLE II-Correlation analysis of oxidants and antioxidants in patients with preeclampsia

	Correlation analysis
Homocysteine vs SOD	-0.55
Homocysteine vs GPx	0.83
Homocysteine vs MDA	0.9
MDA vs SOD	-0.48
MDA vs GPx	0.75

DISCUSSION

Preeclampsia remains a frequent and potentially dangerous complication of pregnancy. It is estimated that approximately 10–15% of maternal deaths are associated with preeclampsia and eclampsia yearly [9] Though the cause is largely unknown, a generalized inflammatory state and oxidative stress are the predominant features of the maternal syndrome. The placental trophoblast NAD(P)H oxidase is the principal source of free radical synthesis. Our study suggests an elevated level of MDA, a marker of free radical generation in patients of preeclampsia. Placenta is rich in polyunsaturated fatty acids and could serve as a rich source of lipid peroxides. Numerous independent studies assessing oxidative damage biomarkers have strengthened our evidence for lipid peroxidation [6,10] in these patients.

Deleterious effects of free radicals include initiation of lipid peroxidation, oxidative damage of biomolecules, and cellular dysfunction, and it is proposed that these may initiate maternal vascular endothelial dysfunction and leukocyte activation, recognized features of preeclampsia. Further, the production of vasoconstrictor endothelin is increased in preeclampsia.[11] However, certain studies are not supportive of a role for oxidative stress in the maternal circulation. [8,12,13] Our study and other studies[14] reveal an increase in the lipid peroxides along with a decrease in the antioxidant enzymes which could cause lipid peroxidation in preeclampsia Sharma et al [15] however demonstrated an increase in the levels of antioxidant enzymes. The decrease in the levels of the antioxidant enzymes could be due to an increase in the utilization of these enzymes to counteract lipid peroxidation. Diminution of the antioxidant response to the oxygenation stimulus results in oxidative stress that may lead to trophoblast degeneration and possibly contribute to impairment of trophoblast invasion and diminished remodeling of the spiral arteries.[16]A defective response to an oxidant stimulus could therefore be one of the earliest events in preeclampsia.

Homocysteine (hcy) is a sulphur containing amino acid, derived from demethylation of methionine, an essential amino acid, requiring folate, vitamin B12 and B6 as co-enzymes. Our findings reveal lower levels of homocysteine in normal pregnancy. The decrease in the levels of homocysteine are generally lower during pregnancy either due to physiological response to pregnancy, increase in estrogen, hemodilution or increased demand for methionine by both mother and the fetus[17]. Murphy et al. [18] demonstrated that the reduction cannot be accounted for by folic acid supplementation, plasma-volume expansion, or a decrease in serum albumin. Low tHcy represents a physiologic adaptation to pregnancy, mediated by endocrine changes. The decrease in homocysteine levels which occurs in normal pregnancy does not occur in preeclampsia. So it is possible that the increase in homocysteine concentration in preeclampsia which is evident in our study is related to the defect in the mechanism that usually decreases homocysteine during normal pregnancy. We could not measure folic acid and vitamin B12 in the study subjects because of cost effectiveness. However, in our study, normal general blood picture ruled out any folic acid deficiency.

Our study demonstrates an increase in the homocyteine levels in patients with preeclampsia. This is in accordance with other studies[19,20,21]. Moreever, in our study a positive correlation is found in between MDA and hcy in patients with preeclampsia. Oxidative stress may be a possible link in between homocysteine and preeclampsia. Vascular damage in the maternal uteroplacental and foetal umbilical placental circulation is supposed to be the central feature though endothelial dysfunction, smooth muscle cell proliferation and coagulation abnormalties, [22,23] also contribute to the development of preeclampsia. These common pathologic mechanism(s) which result in vascular damage serve as an link between hhcy and vascular related pregnancy disorders.[24]

The concentration of tHcy in plasma is a responsive marker of impaired folate status. It has been suggested that maternal hyperhomocysteinemia though a risk factor for placenta-mediated diseases, such as preeclampsia, spontaneous abortion, placental abruption, and recurrent pregnancy loss has a direct adverse effect on the developing fetus. [25,26] Increased tHcy may be a marker of underlying conditions that are directly related to pregnancy complications, such as subclinical vascular disease, reduced glomerular filtration rate [27], and inadequate plasma-volume expansion [28] and it could be directly involved by causing vasculopathy and defective chorionic villous vascularization leading to inadequate maternal–fetal circulation.[21]

Thus in conclusion, in pregnant women the vascular endothelium may be more sensitive to oxidative stress and elevated homocysteine level. This may be resposible for the evelopment of preeclampsia. Hence, there is a need to plan further clinical studies on large scale to understand the association of hhcy and oxidative stress in preeclampsia. along with hcy lowering effect of vitamin B6 , vitamin B12 and folate. There is a possibility that promotion of regular use of B-vitamin and folate by women will be cost effective strategy for the decrease in the micronutrient deficiency related health problems including preeclampsia.

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