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VARIATIONS IN LEVELS OF GAMMA GLUTAMYL TRANSFERASE AND GLUTATHIONE PEROXIDASE DURING PREGNANCY ADVANCEMENT AND PREECLAMPSIA

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ABSTRACT

Background : Oxidative stress is known to be a key factor associated with preeclampsia. Glutathione Peroxidase(GPx), an antioxidant enzyme along with cellular γ -Glutamyl transferase (GGT) is responsible for protecting cells from damage due to free radicals like hydrogen and lipid peroxides.

Aim : To assess prooxidant and antioxidant GGT and GPx in pregnancy and preeclampsia.

Design : A case control study.

Material and methods: Estimation of malondialdehyde, GGT and GPx level in blood was carried out by chemical and enzymatic kit method in 20 non-pregnant women as controls, 20 pregnant women in first week of each trimester and 20 preeclampsia patients.

Statistical analysis: Done by `t` test.

Results and conclusion: Serum GGT though not significant increased in first trimester was found to be towards the upper limit of normal range in pregnant women and raised above normal in preeclampsia patients($p<0.01$). GPx level decreased with progression of pregnancy (p value <0.01) and was found to be decreased in preeclamptic patients. Whether these changes can prove the utility of antioxidants in early pregnancy for the development of preeclampsia needs to be assessed.

Key words: Preeclampsia, Oxidative stress, Glutathione Peroxidase, Gamma glutamyl transferase, Antioxidant.

INTRODUCTION

Preeclampsia is a condition that typically starts after the 20th week of pregnancy. It is characterized by the clinical triad of hypertension, proteinuria and oedema[1]. It is a leading global cause of maternal and infant illness and death. Preeclampsia occurs mostly in women aged 20 to 35. Oxidative stress is inevitable in this condition. This condition can be correlated with the fact that Gamma Glutamyl transferase (GGT) generates reactive oxygen species (ROS) and its serum level is associated with oxidative stress of body [2,3]. GGT plays a key role in the γ-glutamyl cycle, a pathway for the synthesis and degradation of glutathione and also in drug and xenobiotic detoxification[4]. Serum GGT is a known marker of inflammation[5].It is also elevated in diseases of the liver, biliary system, pancreas and congestive heart failure [6,7]. On the other hand, Glutathione Peroxidase (GPx) plays a critical role in protecting the cell from free radical damage, particularly lipid peroxidation. The GPx catalyzes reduction of H_2O_2 to water and organic peroxides (R-O-O-H) to the corresponding stable alcohols (R-O-H) using glutathione (GSH) as a source of reducing equivalents[8]. Thus cells are protected against oxidative stress by extracellular GSH (reduced glutathione). Level of GPx in the body is closely linked with that of glutathione, the master antioxidant. It is thought that increased ROS may be due to decreased GPx in body and these changes may increase GGT. Increased GGT activity in preeclampsia can be viewed as a response to oxidative stress[3]. Since GGT is indirectly involved in detoxifying peroxides produced during oxidative stress which is reported to be induced in pregnancy and preeclampsia[9,10] we attempted to find the relation of MDA, GGT and GPx in pregnancy and preeclampsia.

MATERIALS AND METHODS

A case control study was carried out in the Department of Biochemistry, NKPSIMS, Nagpur for a period of one and a half year. The study protocol was cleared by the ethics committee of the Institute. 20 samples of each group (20-35 years) i.e. pregnant 1st Trimester (TM), 2nd TM, 3rd TM were collected. The subsequent pregnancy outcome in

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the patients was reported to be normotensive. Similarly 20 samples of established preeclampsia were collected. The patients were matched with 20 healthy non-pregnant controls of same age group. An informal written consent was obtained from each subject participating in the study.

Venous blood samples were collected in dry plain tubes from patients and normal healthy controls. Blood samples were centrifuged at 3000 rpm for 10 minutes. Whole blood was collected in heparinised tubes for GPx estimation. Serum collected was stored at -20 °C till estimation. GGT, GPx and MDA levels were estimated in each sample.

Serum GGT activities were determined using Randox diagnostic kits following the principle described by Szasz (1969). Serum malondialdehyde (MDA) estimation method was based on the fact that lipid peroxide condenses with 1-methyl-2-phenyl indole (MPI) 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.

Glutathione peroxidase assay is carried out by the enzymatic kit method by Paglia et. al.[11], 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. Statistical analysis was performed using t-test. The p values were calculated by comparing the results of each group with previous one (eg. III trimester compared with II trimester), and I trimester is compared with controls. This was done with the aim of finding variations of the values in pregnancy advancement.

RESULTS

As seen in table I the levels of GGT increased from the second trimester in normal pregnant women. The values were significantly increased ($p<0.01$) in preeclampsia women when compared with the women in third trimester (table II). Also the levels of GPx were significantly decreased ($p<0.01$) when normal pregnant women in I, II and III trimester were compared with women with preeclampsia (table II and III). The levels of MDA was significantly increased ($p<0.01$) in normal pregnant women in third trimester when compared with preeclampsia women (Table II). Also the levels of MDA were significantly increased when women in the I and II trimester were compared with normal healthy controls.

Table I : Concentration of serum GGT, GPx and MDA at various stages of pregnancy and preeclampsia.

Parameter	Non pregnant (control) (n=20)	Normal Pregnant women			Preeclampsia (n=20)
		1st Trimester (n=20)	2nd Trimester (n=20)	3rd Trimester (n=20)	
GGT (U/L)	20.25 ± 3	23±4	33.9±3	41.55±2	47±2
GPx (U/L)	5600±1142	4801±258	4596±411	4557±311	3986±182
MDA (nmol/ml)	0.945± 0.33	1.54±0.29	1.73±0.21	2.255±0.22	5.25±1.17

Table II : Concentration of serum GGT, GPx and MDA in III trimester pregnancy women and preeclampsia

Parameter	Normal Pregnant women	Preeclampsia (n=20)
	3rd Trimester (n=20)	
GGT (U/L)	41.55±2	47±2*
GPx (U/L)	4557±311	3986±182*
MDA (nmol/ml)	2.255±0.22	5.25±1.17*

P<0.01 when normal pregnant women in III trimester are compared with preeclamptic females

Table III : Concentration of serum GGT, GPx and MDA in I and II trimester normal pregnancy and normal healthy controls

Parameter	Non pregnant (control) (n=20)	Normal Pregnant women	
		1st Trimester (n=20)	2nd Trimester (n=20)
GGT (U/L)	20.25 ± 3	23±4	33.9±3*
GPx (U/L)	5600±1142	4801±258*	4596±411*
MDA (nmol/ml)	0.945± 0.33	1.54±0.29*	1.73±0.21*

*P<0.01 when non pregnant women are compared with normal pregnant women in I and II trimester.

DISCUSSION

GGT is an important enzyme in metabolism of extracellular GSH. It is present in the cell membrane of liver and many other tissues. It catalyzes the transfer of the γ-glutamyl moiety of glutathione to an acceptor that may be an amino acid, a peptide or water (forming glutamate)[12,13]. Increased GGT level is a reflection of excessive use of GSH and GSH is overused when free radical production in the body is in excess. This occurs mostly in hypertension, lipid peroxidation, inflammation, drug toxicity etc. which are the causes of oxidative stress. The reaction is catalyzed by GPx where GSH destroys peroxides and gets utilized in the process. Hydroperoxides (ROS-reactive oxygen species) produced during the stress are detoxified. Extracellular GSH of the cell is consumed in the process. GGT induction can occur as a protective adaptation that allows cells access to more cysteine and thereby increases intracellular glutathione, which is protective against oxidative stress. Hence GGT is expected to be increased as it is responsible for the homeostasis of GSH in normal pregnancies also. But GGT activity is found to be normal in normal pregnancy in most published studies [14-15]. Bacq et. al.[16] reported GGT activity which was significantly lower during the second and third trimesters compared with nonpregnant controls. Girling JC states that GGT can be a marker of oxidative stress [10] which is reported to be induced in normal pregnancy and rises quite high in preeclampsia[9]. In our study, serum GGT levels are normal in 1st trimester pregnant women while it is at the upper limit of normal range in samples of 2nd and 3rd trimester of normal pregnant women. The raise in GGT levels in serum samples of preeclampsia patients ($p<0.01$) supports above observation and our hypothesis. But to establish GGT as marker of preeclampsia a follow-up study with normal and preeclampsia pregnancy is essential. The condition of preeclampsia is not sudden in third trimester. The initial development of oxidative stress on onset of pregnancy must be slowly progressing into preeclamptic condition. It is thought that if there is any biochemical change in these patients which is detectable in early stage, it would help in controlling further complications in early pregnancy might be indicative of future development into oxidative stress and preeclampsia.

GPx concentration also becomes important in these circumstances. The findings of Baotet et al [17] show significantly higher levels of GPx in both fetal and maternal circulations of the preeclamptic group indicating that preeclampsia is associated with a specific antioxidant. Similarly levels of MDA, GPx in severe and mild Preeclampsia pregnant women were significantly higher than healthy pregnant women as per the observations of Mostafa et al [18]. On the contrary the study of Hiten Mistry et.al.[19] revealed highly significant reductions in serum selenium concentrations and plasma glutathione peroxidase activity in pregnancy ($p<0.01$) per se compared to nonpregnant controls. In our samples GPx concentration was found to decrease ($p<0.01$) in preeclampsia patients. The decrease in GPx level in our reports may be one of the reasons of extensive raise in oxidative stress in preeclampsia.

Increased levels of MDA and reduced enzymatic antioxidants activities are already reported in pregnant women with preeclampsia demonstrating the presence of oxidative stress [20,21]. It is hypothesized that intermittent placental perfusion, secondary to deficient trophoblast invasion of the endometrial arteries, leads to an ischemia–reperfusion-type insult and results in the generation of free radicals.[21] In our study also a significant increase in the serum MDA levels ($p<0.01$) ensures the increase in ROS in pregnancy and significantly high in preeclampsia.

Thus, GGT and GPx are fluctuating in pregnancy and in preeclampsia. Tendency of increase in GGT activity is observed as pregnancy advances. In preeclampsia, GPx is decreased while GGT is raised, though not significantly. These changes are apparently due to raised ROS.

CONCLUSION

Follow up studies are required to correlate the value of glutathione, GPx, GGT concentrations in blood of preeclamptic patients to assess whether GGT can be used as a marker in pregnant women for the early diagnosis of preeclampsia.

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