CORRELATION BETWEEN LIPID PEROXIDATION AND NON-ENZYMATIC ANTIOXIDANTS IN PREGNANCY INDUCED HYPERTENSION

Sadanand B Patil, M V Kodliwadmth and Sheela M Kodliwadmth*

Departments of Biochemistry and *Obstetrics and Gynaecology, J.N. Medical College, Nehru Nagar, Belgaum-10

ABSTRACT

The present study was designed to evaluate the lipid peroxidation and non-enzymatic antioxidants in pregnancy-induced hypertension (PIH) compared with healthy normal pregnant and non-pregnant as controls. 75 cases were studied of which 25 were normal healthy controls (Group A), 25 healthy pregnant women (Group B) of third trimester and 25 were PIH (Group C) of the same trimester. In PIH, malondialdehyde a lipid peroxidation product was significantly increased as compared to normal pregnant and non-pregnant controls. The non-enzymatic antioxidants like reduced glutathione, Vitamin-E, Vitamin-C and Vitamin-A were significantly decreased in normal pregnant as compared to controls. A further significant decrease was noticed in PIH compared to normal pregnant. A significant negative correlation was detected between lipid peroxidation and non-enzymatic antioxidant levels. Our study clearly indicates a relationship between elevated lipid peroxidation, decreased non-enzymatic antioxidant in PIH. The measurement of non-enzymatic antioxidants in plasma may be useful predictor of the likely development of PIH.

KEY WORDS

Lipid peroxidation, Malondialdehyde, Oxidative stress, Pregnancy Induced Hypertension.

INTRODUCTION

Pre-eclampsia is a pregnancy complication recognized by new-onset gestational hypertension and proteinuria. The disorder affects mothers and their infants. Once the disease is evident clinically, it can be cured only by delivery (1). Pre-eclampsia is a pregnancy specific syndrome. It occurs approximately 5% of all pregnancies, 10% of first pregnancies and 20-25% of women with chronic hypertension (2). Lipid peroxidation is an oxidative process that normally occurs at low levels in all cells and tissues. Under normal conditions, a variety of antioxidant mechanisms serve to control this peroxidative process (3). However, a diminution of normal antioxidant function will allow an increase of peroxidative activity to occur at expense of oxygen and polyunsaturated fatty acids. In disease states such as toxemia of pregnancy, an imbalance between lipid

peroxidation and antioxidant mechanisms could impair normal endothelial function. Sera lipid peroxidation products are increased in pregnant women and this increase is further augmented in toxemic patients with decreased antioxidant levels (4).

The aim of the present study is to find out the biochemical changes in respect to lipid peroxidation and non-enzymatic antioxidant defenses (reduced glutathione, Vitamin-E, Vitamin-C and Vitamin-A) in normal pregnant women and PIH of third trimester and non-pregnant healthy controls.

MATERIALS AND METHODS

The present study was carried out jointly by the Department of Biochemistry and Obstetrics and gynecology during July 2000 to June 2004. The ethical committee of J.N.Medical College and district civil hospital Belgaum approved the study protocol. Informed consent was taken from individual subjects. The study comprises of 75 cases, of which 25 were normal healthy controls (Group A), 25 healthy third trimester pregnant women (Group B) and 25 were third trimester PIH (Group C). The subjects selected for the present study were attending and admitted to District Civil Hospital, Belgaum, whose age

Address for Correspondence :

Dr. Sadanand B.Patil

Department of Biochemistry

J.N.Medical College Nehru Nagar

Belgaum-5900010 Karnataka

E-mail : sanrum03@yahoo.co.in

ranged from 20-40 years. The diagnosis of pre-eclampsia was based on the definition of American College of Obstetrics and Gynecologists (5) 1. Systolic blood pressure greater than 140 mm Hg or a rise of at least 30 mm Hg or 2. Diastolic blood pressure greater than 90 mm Hg or a rise of at least 15 mm Hg (manifested on two occasions at least 6 hours apart), and 3. Proteinuria of 300 mg or greater in a 24 hour urine collection or protein concentration of 1 gm/ Ltr (on two occasions at least 6 hours apart). In the control group no one was deficient in non-enzymatic antioxidants. Where as in third trimester pregnant 21 were deficient in reduced glutathione, 23 in Vitamin-E and 12 in Vitamin-C and A. In case of third trimester PIH patients all 25 were deficient in reduced glutathione, and Vitamin-E, 21 in Vitamin-C and 20 in Vitamin-A. Group individuals did not receive any supplementation of antioxidant vitamins and none of the women had received antihypertensive medication until the study samples were taken. The levels of blood pressure and proteinuria were determined at the time of sampling.

All the subjects were of low socio-economic status as they had low income. The obesity, diabetes mellitus under medication and untreated diabetes, alcoholic, severe anemia (<6.0 gm% of Hb) and suffering from any other systemic disorders were excluded from the present study.

5 ml of blood sample was drawn by venipuncture and collected in a heparinized tube. Malondialdehyde, a product of lipid

peroxides detectable in blood, was used as an indicator of lipid peroxidation. Malondialdehyde concentrations were determined by using Thiobarbituric acid (6). Plasma was separated to determine the antioxidant levels like Reduced glutathione (7), Vitamin-E (8), Vitamin-C (9) and Vitamin-A (10). Statistical data were expressed as Mean $\pm$ SD (standard deviation), median, range and statistical significance was determined by ANOVA and Bonferroni multiple comparison test.

RESULTS AND DISCUSSION

A statistically significant increase in the levels of circulating malondialdehyde a marker of lipid peroxidation was observed in third trimester normal pregnant women as compared non-pregnant controls. Further increase was observed in PIH patients when compared to normal pregnant and non-pregnant controls (Table 1). Activation of PGH synthase can generate oxygen radicals that could act on lipids to generate increased lipid peroxides in normal pregnant, which will further aggravate during PIH (11).

Antioxidants oppose toxic actions of lipid peroxides and oxygen radicals and limit the amount of lipid peroxides that are formed. Decreasing trend of all the non-enzymatic antioxidants like reduced glutathione, Vitamin-E, Vitamin-C and Vitamin-A were observed in third trimester normal pregnant women as compared to non-pregnant controls. Further decrease was

Table 1: Malondialdehyde (MDA) and non-enzymatic antioxidant (Reduced glutathione, Vitamin-E, Vitamin-C, Vitamin-A) levels in the non-pregnant, normal pregnant of third trimester and PIH patients

Parameters		Group A (Non-pregnant) n=25	Group B (Third Trimester pregnant) n=25	Group C (Third Trimester pre-eclamptic) n=25	Statistical Relation	
					F-value	P-value
MDA (nmol/ml)	Mean $\pm$ SD	1.19 $\pm$ 0.09	1.79 $\pm$ 0.14	2.93 $\pm$ 0.54	175.734	Group A – Group B $\rightarrow$ 0.000 Group A – Group C $\rightarrow$ 0.000 Group B – Group C $\rightarrow$ 0.000
	Median	1.21	1.86	2.95		
	Range	(1.04 – 1.38)	(1.41 – 1.92)	(1.83 – 4.01)		
Reduced-glutathione (mg%)	Mean $\pm$ SD	59.97 $\pm$ 1.30	50.77 $\pm$ 6.91	41.35 $\pm$ 4.23	93.343	Group A – Group B $\rightarrow$ 0.000 Group A – Group C $\rightarrow$ 0.000 Group B – Group C $\rightarrow$ 0.000
	Median	59.64	50.77	41.54		
	Range	(58 – 62)	(39.62 – 64.32)	(32.31 – 50.77)		
Vitamin-E (mg%)	Mean $\pm$ SD	1.42 $\pm$ 0.19	0.87 $\pm$ 0.15	0.57 $\pm$ 0.16	159.744	Group A – Group B $\rightarrow$ 0.193 Group A – Group C $\rightarrow$ 0.000 Group B – Group C $\rightarrow$ 0.000
	Median	1.36	0.89	0.50		
	Range	(1.19 – 1.81)	(0.52 – 1.11)	(0.40 – 0.93)		
Vitamin-C (mg%)	Mean $\pm$ SD	1.11 $\pm$ 0.24	0.96 $\pm$ 0.33	0.60 $\pm$ 0.24	21.911	Group A – Group B $\rightarrow$ 0.000 Group A – Group C $\rightarrow$ 0.000 Group B – Group C $\rightarrow$ 0.000
	Median	1.00	0.83	0.50		
	Range	(0.66 – 1.66)	(0.33 – 1.50)	(0.33 – 1.17)		
Vitamin-A (μ g%)	Mean $\pm$ SD	28.62 $\pm$ 3.45	22.84 $\pm$ 5.07	18.93 $\pm$ 5.28	26.242	Group A – Group B $\rightarrow$ 0.000 Group A – Group C $\rightarrow$ 0.000 Group B – Group C $\rightarrow$ 0.015
	Median	28.8	21.8	17.7		
	Range	(20.3 – 36.2)	(14.4 – 31.8)	(11.5 – 30.3)		

noticed in PIH when compared to normal pregnant and non-pregnant controls (Table 1).

Oxidative stress during pregnancy and PIH was evaluated in the present study by analyzing pro-oxidant and non-enzymatic antioxidants. Lipid peroxidation was considered as a marker for pro-oxidant, whereas reduced glutathione, Vitamin-E, Vitamin-C and Vitamin-A were considered as antioxidant. Free radicals by their unstable and transient nature are difficult to measure directly. Their tendency to cause lipid peroxidation has been used as an indirect measure. Markers of lipid peroxidation (MDA) have been increased during the progression of normal pregnancy (12). Non-enzymatic antioxidants like reduced glutathione, Vitamin-E, Vitamin-C and Vitamin-A oppose the toxic actions of lipid peroxides and oxygen radicals and limit the amount of lipid peroxide formation. In the lipophilic environment tocopherol acts as protective chain breaking antioxidant. Vitamin-C acts as antioxidant by scavenging superoxides, hydroxyl radicals and various lipid hydroperoxides. In addition it can also restore the antioxidant properties of oxidized Vitamin-E.

A significant increase in lipid peroxides (MDA) levels was observed in third trimester of normal pregnant compared to non-pregnant in present study, further increase was observed in PIH patients when compared to normal pregnant as well as non-pregnant. Increased lipid peroxidation is correlated with increased free radical formation. Results of the present study confirm increased oxidative stress during normal pregnancy and PIH. Lipid peroxidation may be an important factor in the pathogenesis of PIH (15).

Hubel, et al, and Kharb, et al have also shown that serum lipid peroxides are known to increase in pregnancy and this increase was exaggerated in pre-eclampsia. This increased lipid peroxides level can increase the susceptibility of polyunsaturated fatty acid to peroxidative damage, presumably by free radicals that may lead to the formation of malondialdehyde (13, 14).

Glutathione is an effective reductant and plays an important role in a variety of detoxication processes. It readily neutralizes the hydroxyl radicals, which are considered as a major source of oxidative stress. Decreased levels of glutathione in maternal blood might indicate decreased detoxication of free radical scavenging in pre-eclampsia (16). Our study showed a significant decrease in the level of reduced glutathione and increased levels of malondialdehyde in PIH as compared to normotensive pregnant as well as non-pregnant controls (17).

Kharb reported that vascular endothelial damage has been implicated in the pathophysiology of pre-eclampsia. Significantly lowered levels of Vitamin-E and Vitamin-C were observed in pre-eclamptic women as compared to controls (18). Our study indicates that normal pregnant women had markedly reduced plasma alpha tocopherol, ascorbic acid, and retinol as compared to non-pregnant controls. Further decrease was observed in PIH patients when compared to normal pregnant. Our findings exhibit markedly increased levels of lipid peroxidation and significantly decreased levels of non-enzymatic antioxidants are important factor in the pathogenesis of PIH (19, 20, 21).

The present study clearly indicates that significant increase in malondialdehyde with diminished level of non-enzymatic antioxidants like reduced glutathione, Vitamin-E, Vitamin-C and Vitamin-A are responsible for the pathogenesis of PIH. Early attention, intensive management and better treatment with antioxidant vitamins may be essential for improving the maternal and foetal outcome in PIH.

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