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Increased level of Lipid peroxidation in preeclamptic pregnancy; a relationship with paraoxanase 1(PON1) activity.

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Abstract

The aim of the study was to evaluate the status and diagnostic utility of PON1as indicator of antioxidant status in preeclampsia. Study groups involved cases, women with preeclampsia (BP >140/90mmHg, edema and proteinuria) (n=30) and controls, pregnant women without preeclampsia or any other complications attending hospital for antenatal checkup (n=30). Both cases and control groups were not on any vitamin E/ vitamin C supplementation. Fasting venous samples of blood were collected from both cases and controls. Serum PON1 and serum malondialdehyde (MDA) levels were measured spectrophotometrically. Students independent't' test and Pearsons correlation were used for statistical analysis. We found that PON1 activity in preeclampsia cases were significantly lower than in normal pregnancy controls while MDA levels were significantly higher. Conclusion: Our Study showed that the increase in Oxidative stress in preeclampsia cases is associated with a decrease in PON1 antioxidant activity.

Key Words: Preeclampsia, Paraoxonase

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Introduction

Human serum Paraoxonase (PON1) is an enzyme bound to high density lipoprotein (HDL) that hydrolyses oxidized phospholipids and inhibits low density lipoprotein (LDL) oxidation. It is the major determinant of antioxidant action of HDL.Oxidative stress has been implicated in the pathogenesis of several complication of human pregnancy including preeclampsia. Preeclampsia is a pregnancy specific disorder and its etiopathogenesis is complex and incompletely understood. Preeclampsia is associated with elevated lipid peroxidation and reduced antioxidant status.Preeclampsia is an endothelial disease with a major involvement of lipid mediated oxidative damage.[1] A consistent positive association between maternal dyslipidemia and the risk of preeclampsia have already been found.

MDA is the end product of lipid peroxidation and reflects the oxidative status of the biological system [2]. MDA causes damage to LDL molecules. The altered LDL is taken up by macrophages via scavenger receptors and forms foam cells. [3] This in turn results in atherogenesis.

PON1 (E.C 3.1.1.2) an esterase enzyme associated with HDL exerts a protective effect against oxidative damage of circulating cells and lipoproteins. [4] The hydrolysis of the oxidized phospholipids by PON destroys the biologically active lipids in mildly oxidized LDL. [5] Thereby, HDL and its associated PON1 interrupt a process that would otherwise lead to oxidative damage. PON1 thus can be an indicator of antioxidant status in preeclampsia. Oxidative stress is known to be increased in preeclampsia. Hence this study was aimed to assess the PON 1 activity and MDA levels in preeclampsia along with the Lipid profile and to find their correlation.

Materials and Methods.

The study population consisted of about 60 pregnant women who visited Dr.B.R.Ambedkar Medical College and Hospital, This study was a descriptive study and women with and without preeclampsia was recruited consecutively. The study protocol was approved by the Institutional Ethics Board.

30 pregnant women with preeclampsia were included in the study. The diagnosis of preeclampsia was based on clinical examination; raised blood pressure (BP>-140/90mmHg), edema and examination of urine for proteinuria by dipstick method. 30 normal pregnancies with no preeclampsia or any other complications were included as controls. The exclusion criteria included patients those on vit C/ vit E or antioxidant supplementation or with any other complications other than preeclampsia like gesta-

tional diabetes, diabetes mellitus, hypertension, coronary heart disease, impaired renal function, multiple pregnancy and other chronic diseases that might interfere with the study.

Laboratory Assay

Fasting venous blood samples were collected, the sample was centrifuged at 3000rpm for 10 minutes and serum was separated as early as possible and stored for analysis. Paraoxonase activity was determined by measuring the rate of hydrolysis of paraoxon (dimethyl –p-nitrophenylphosphate) by monitoring the increase of absorbance at 412nm at 37^0c. The amount of generated p-nitrophenol was calculated from the molar absorptivity coefficient at a pH of 8.5, which was 18290 $mol^{-1}cm^{-1.}$ [6]PON activity was expressed as U/L serum. One Unit (U) of paraoxanse activity was defined as one micro mol of released p-nitrophenol per litre of serum per minute and expressed in U/L [7]

Serum MDA was estimated using 40% trichloroacetic acid (TCA) and 0.67% thiobarbituric acid (TBA) and absorbance was recorded at 530nm. The MDA content was calculated using the molar extinction coefficient 1.56 x 10^5 and expressed as nmoles/ dl [8].S total cholesterol, triglyceride and HDL –C were assayed with BA300 automated chemistry analyzer by using commercially available kits (Diasys diagnostics). The concentration of LDL was calculated using Friedewalds formula.

All statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) software version15.0, The comparison of parameters were performed using students 't' test(two tailed, independent) and correlation analysis were performed using Pearsons correlation test.

Results

The demographic features and clinical data of pregnant women with and without preeclampsia are summarized in table 1and 2.The two groups were matched in terms of age and BMI in table 1 and with regards to Gravida in table 2. There was no significant difference in women's with and without preeclampsia in regards to number of pregnancies.

Table 1*.The demographic features of pregnant women with and without preeclampsia.*

Basic variables	Pregnancy Controls	Preeclampsia Cases
Age in years	24.20±3.45	26.73±5.21
BMI (kg/m^2)	23.96±1.69	27.69±2.41

Table 2. *Gravida distribution.*

Gravida	Pregnancy Controls	Preeclampsia Cases
Gravida I	9 (30.0%)	5(16.7%)
Gravida II	12(40.0%)	11(36.7%)
Gravida III & above	9(30.0%)	14(46.7%)
Total	30(100.0%)	30(100.0%)

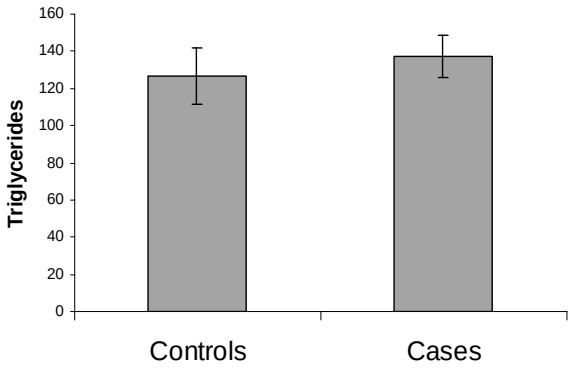

Figure 1. *Serum Triglyceride (mg/dl) in pregnancy controls and preeclampsia cases.(Mean ±S.D)*

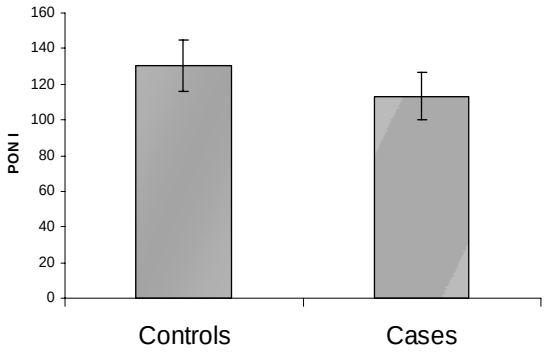

Figure 2. *PON1 activity(U/l) in pregnancy controls and preeclampsia cases (Mean ± S.D)*

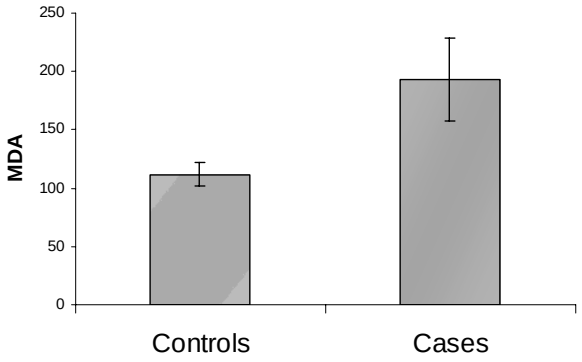

Figure 3. *Serum MDA levels (nmoles/dl) in pregnancy controls and preeclampsia cases.(mean ±S.D)*

***Table 3**: Comparison of Study variables in Normal pregnancy Controls and Preeclampsia Cases (Mean ±S.D)*

Variables	Pregnancy Controls	Preeclampsia Cases	Significance
PON-I U/L	130.72±14.44	113.31±13.01	t=4.906;p<0.001**
MDA nmoles /dl	111.91±10.17	193.19±35.68	t=11.999;p<0.001**
Triglycerides mg/dl	126.76±15.17	137.55±11.45	t=3.110;p=0.003**
Total cholesterol mg/dl	149.12±10.72	161.75±14.53	t=3.830;p<0.001**
LDL mg/dl	90.36±11.94	103.03±14.71	t=3.664;p=0.001**
HDL mg/dl	33.05±3.56	31.21±3.53	t=2.008;p=0.049*
LDL/HDL ratio	2.76±0.62	3.36±0.77	t=3.352;p=0.001**
PON-I/HDL ratio	3.96±0.67	3.68±0.58	t=1.756;p=0.084+

***Table 4.** Correlation of MDA with study variables*

Pair	Preeclampsia Cases	
	r value	p value
MDA vs Triglycerides	-0.177	0.350
MDA vs Total cholesterol	0.187	0.322
MDA vs LDL	0.231	0.219
MDA vs HDL	-0.080	0.674
MDA vs LDL/HDL ratio	0.183	0.332
MDA vs PON- I/HDL ratio	0.117	0.539

The total cholesterol (t=3.830; p< 0.001) and triglycerides (t=3.110; p=0,003) were significantly higher in preeclampsia women as compared to the other group [Figure 1]. The LDL level was significantly higher in women with preeclampsia than in pregnant women without preeclampsia (t= 3.664; p=0.001). In contrast the HDL level was significantly lower in women with preeclampsia in comparison with pregnant women without preeclampsia (t=2.008; p=0.049). PON1 activity was significantly lower in women with preeclampsia than in pregnant women without preeclampsia (t= 4.906; p<0.001) where as MDL level and LDL /HDL ratio were significantly higher (t=11.999; p<0.001, t=3.352; p=0.001 respectively). [Figure 2 and Figure 3.] . In addition the PON1 / HDL ratio did not differ significantly between pregnant women with and without preeclampsia [Table 3].

***Table 5.** Correlation of PON-I with study variables*

Pair	Preeclampsia Cases	
	r value	P value
PON-I vs MDA	0.063	0.743
PON-I vs Triglycerides	0.135	0.477
PON-I vs Total cholesterol	-0.023	0.906
PON-I vs LDL	-0.057	0.765
PON-I vs HDL	0.057	0.766
PON-I vs LDL/HDL ratio	-0.075	0.695
PON-I vs PON- I/HDL ratio	0.632	<0.001**

In women with preeclampsia PON-1 activity correlated positively with PON-1/HDL ratio (r= 0.632,p<0.001), triglyceride levels (r=0.135, p= 0.477) and HDL levels (r=0.057;p=0.766) while total cholesterol level (r=-0.023 ;p=0.906) and LDL levels (r= -0.057 ;p=0.765) was inversely correlated (Table 3). The MDA levels were positively correlated in preeclampsia women with total cholesterol (r= 0.187; p=0.322), LDL (r= 0.231; p=0.219), LDL/HDL ratio (r=0.183; p= 0.332) and PON-1 / HDL ratio (r=0.117; p=0.539). In addition there was inverse correlation between MDA and triglyceride level (r= -0.177; p= 0.350) and HDL levels (r= -0.080; p=0.674) [Table 4}.

PON-1 Activity also correlated positively with MDA levels in preeclampsia women (r= 0.063; p=0.743) though not statistically significant [Table 3].

Discussion

There are several proposed mechanisms for explaining the anti atherogeneic properties of HDL. PON1 is calcium dependent esterase, is exclusively bound to the HDL fraction of serum. [9]. PON1 prevents the oxidative modification of LDL and is responsible for the antioxidant activity of HDL [2]. Preeclampsia has been associated with atherogenic wall changes in the uteroplacental bed.[10] These changes consequently results in necrosis of vessel wall and accumulation of lipid laden foam cells with oxidized LDL.In preeclampsia, the placental damage is progressive and can be compensated for sometime depending on the severity of initial placental defect and intrinsic placental antioxidant capacity.[11]

Lipid peroxides are directly involved in mediating maternal endothelial dysfunction by increasing the production of thromboxane A2 and the expression of cell adhesion molecules in the uteroplacental vasculature as well as in the maternal peripheral vasculature.[12] Maternal plasma lipids are significantly elevated during pregnancy. In preeclampsia dyslipidemia pattern of increased concentra-

tion of triglyceride, cholesterol and LDL and decreased concentration of HDL have been noticed. Peroxidation of lipids brings about changes in its molecular structure and these changes becomes more marked when the damaged lipids are the constituents of biological membrane Low antioxidant levels may aggravate prooxidant injury on endothelial cells, altering prostacyclin – thromboxane balance and culminating in preeclampsia.[13],[14]. Hence mechanisms that prevent oxidation of LDL have received increased attention in recent years. One such mechanism is prevention of LDL oxidation by PON1.

In the current study it was found that the PON1 activities were significantly lower in women with preeclampsia than in women with normal pregnancy. MDA levels were significantly higher in preeclampsia cases. The increased MDA levels in preeclampsia is known to be due to increased generation of reactive oxygen species and increased oxygen demand along with reduction in activities of enzymes like superoxide dismutase, glutathione peroxidase and decrease in concentration of antioxidants like Vitamin C and Vitamin E.Reactive oxygen species can cause enhanced lipid peroxidation.

Uzun, Benian, Madazh, Topcuoglu, Aydin, Albayrak [15] have reported oxidative stress and decreased paraoxonase activity in preeclampsia. Decreased PON1 activity can cause damage to the vascular endothelium. Preeclampsia results in an increased vascular oxidative stress and endothelial damage; although it is not very clear whether this phenomenon occurs before or after the development of preeclampsia. PON1 modulates the susceptibility of HDL to atherogenic modifications such as oxidation, glycation and homocysteinylation and even exerts an anti-inflammatory role.

In Summary, this study has found increased MDA levels and decreased PON1 activity in preeclampsia. The decrease in PON1 activity is associated with decreased HDL levels. It is not very clear whether PON1 activity reduction is a consequence of decreased HDL levels or increased oxidative stress. Further studies are required to substantiate the postulated correlation between the lower paraoxonase activity level and pathophysiology of preeclampsia.

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