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Human Monocytic Paraoxonse2 (PON2) Association with Birth weight In Preeclamptic Patients

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Abstract

Objective: Intracellular antioxidant enzyme paraoxonase 2 (PON2) may have a protective function in the prevention of atherogenesis. Pre-eclampsia and atherosclerosis are both endothelial diseases with an involvement of lipid-mediated oxidative damage.
Design: Study designed to investigate human monocytic paraoxonase2 activity in preeclampsia and its association with birth weight. We conducted a case-control study.
Methods: Maternal serum was used to measure paraoxonase 2, Nitric oxide and lipid profile.
Population or Sample: 58 women with preeclampsia and 58 with uncomplicated pregnancy.
Main Outcome Measures: PON2 lactonase activity was positively correlated with Birth weight.
Results: Monocytic Paraoxonase2 activity was significantly lower in women with preeclampsia compared with controls (1.228U/mg versus1.645U/mg protein, p =0.003). Serum levels of total cholesterol, LDLc and VLDLc are

significantly higher in cases than in controls. However serum HDLc levels are decreased significantly in cases compared with control group. Serum nitric oxide also showed significant decrease in cases 22.968umol/L versus 24.957umol/L, p = 0.015. There is significant positive association is found in linear regression analysis (Multiple R=0.2, p value=0.035) between PON2 and Birth weight. PON2 lactonase activity was negatively correlated with serum cholesterol (correlation coefficient r=-0.2, p value=0.036). Multivariate logistic regression analysis Model I:, total cholesterol, HDL-C, LDL-C, nitric oxide (R2=0.141, p= 0.012), model II All parameters in Model I + PON2 lactonase activity. (R2 =0.209, p=0.001).

Conclusions: Present study shows PON2 lactonase activity is positively correlated with Birth weight, which also add up the diagnostic predictability of preeclampsia.

Introduction

Pre-eclampsia is a multisystem disorder of unknown aetiology characterised by development of hypertension with proteinuria after the 20week of pregnancy in previously normotensive, non-proteinuric patient [1]. Oxidative stress during pregnancy contributes to diminished placental blood flow and causes hypoxia [2]. Paraoxonases are a family of three enzymes known as PON1, PON2 and PON3, whose genes are located adjacent to each other on chromosome 7q21–22. PON2 is an intracellular protein which protects cells against oxidative damage [3]. PON2 has lactonase activity it is expressed in cells of the artery wall including endothelial cells, macrophages, also predominantly expressed in monocytes and influence lipoprotein properties and cellular oxidation [4]. Plasma lipids and monocytes are important component in blood that contribute to atherogenesis [5]. Major contributors to atherosclerosis are oxidative damage and endoplasmic reticulum (ER) stressinduced apoptosis; which can be diminished by the PON2 [6]. Taking the deleterious effects of oxidized lipoproteins on endothelium and PON2 enzymes protective effect on lipoprotein oxidation into consideration, this study was designed to investigate serum paraoxonase 2 activities and pre-eclampsia and its association with birth weight. This evolutionary perspective raises the question of establishment of interlink between PON2, and NO (Nitrate + Nitrite) in pathophysiology of foetal compromise in preeclampsia. Early identification of LBW babies provides better prognosis. Some biochemical and ultrasonographic parameters have shown promising predictive performance, but so far there is no clinically validated screening procedure for low birth weight.

Material and Methods

This is a hospital based case control study. Total 116 primigravida pregnant females enrolled in this study. 58 female patients diagnosed as having mild Pre-eclampsia admitted to Medical college Hospital, were selected as cases for this study. It is defined as denovo hypertension (140/90 mmHg) measured on two occasion each 6

hours apart appearing after 20 weeks of gestation accompanied by proteinuria (0.3g/24hr). Control population consisted of 58 healthy pregnant females matched for age, gender attending the routine health check-up in our outpatient department. Controls selected on the basis of a negative medical or complicated obstetric history.
None of the women from cases and control had a positive medical history of cardiac and metabolic disease.
The sample size calculation was based on type I alpha error of 5% and a test power of 80%. No participants smoked, used caffeine or alcohol, and had history of thyroid disease, diabetes mellitus, and hypertension. Exclusion criteria included multiple pregnancies, maternal chronic disease (hypertension, endocrine diseases, connective tissue diseases, thrombophilies, acute or chronic hepatic diseases).
Fasting blood samples obtained from antecubital veins of the subjects in the patient and control groups. Fasting venous blood sample of 5 ml collected in the morning from the pre-eclampsia group immediately after the diagnosis before giving any medication and from normal pregnant women at their routine prenatal visits. Two millilitres of blood was transferred into heparinised tube for monocyte extraction using monocyte separation media. The remaining blood was allowed to clot at room temperature in plain bulb for one hour and serum was collected by centrifugation at 1500xg for 10 minutes which was then used for estimation of nitric oxide and lipid parameters. Serum analytes were estimated by ERBA Smartlab auto analyser. Analysis was performed within 24 hours of sample collection kept in freezer compartment till analysis. All chemicals used were of reagent grade. All women gave informed written consent to participate in the study, which had been approved by the institutional Ethics Committee. Serum nitric oxide (nitrate+nitrite) estimation was done as described previously [7]. Lysed monocyte protein estimation were performed using Lowry's method [8]. Lipid parameters were done using routine laboratory method. Mononuclear cells are separated from whole blood using monocyte separation media purchased from Himedia. Monocyte Pon2 Lactonase activity was done as described previously [9]. Monocytic PON2 lactonase activity expressed as U/mg protein.

Statistical Analysis

The results obtained in the study were evaluated using MYSTAT STATISTICAL PCKAGE at 95% confidence interval and at a significance level of $p<0.05$. Results are presented as mean± standard deviation. The continuous variables are tested for normality with Shapiro-Wilk test. Student's unpaired t test used for statistical analysis between cases and controls for numerical variables in Gaussian distribution. The strength of association between two parameters is expressed by the Pearson's correlation coefficient. The logistic regression analysis is used for prediction of risk of pre-eclampsia contributed by various risk factors. The two models prepared in the logistic regression for the analysis of data are as follows.
Model I: total cholesterol, HDL-C, LDL-C, nitric oxide.
Model II: All parameters in Model I + PON2 lactonase activity.

At each step, variable in the model is assessed for its contribution to the model. That was reflected by the Naglekerke R2 value and p value of the model. $p<0.05$ was considered as statistically significant.

Results:

There were no differences in maternal characteristics between the two groups; with regard to age, number of pregnancies and delivery type all are primigravida with normal delivery. Mothers participating in the study were predominantly, 20–35 years old. Serum levels of total cholesterol, Low density lipoprotein-cholesterol and very low density lipoprotein cholesterol are higher in cases than in controls and are statistically significant. However serum HDL-c levels are decreased significantly in pre-eclampsia patients when compared with control group. Serum nitric oxide (Nitrate+Nitrite) also showed significant decrease in cases (22.96±4.83umol/L) as compared to control group (24.96±4.99umol/L), p value=0.031. Monocyte PON2 also showed significant decrease in cases (1.23± 0.81U/mg protein) as compared to control group (1.64±0.69 U/mg protein) p value=0.003 (Figure 1). Birth weight is significantly decreased in cases (2.52±0.62kg) as compared to (2.92±0.42kg) p value< 0.001(Table 1.). There is significant positive association is found in linear regression analysis (Multiple R=0.2, p value=0.035) between PON2 and Birth weight. PON2 lactonase activity is found to be negatively correlated with serum cholesterol (correlation coefficient r=-0.2, p value=0.036).

Multivariate logistic regression analysis used for prediction of risk of pre-eclampsia contributed by various risk factors. At each step, variable not in the model is assessed for its contribution to the model reflected by the Naglekerke R2 value and p value of the model. The two models prepared in the logistic regression for the analysis. Multivariate logistic regression analysis after adjustment of other established risk factors for preeclampsia demonstrates that decreased PON2 lactonase activity is associated with greatest risk for the occurrence of low birth weight. Table2 shows **Model I** (**R2=0.141, p=0.012)** Area under ROC curve: 0.692 (Figure 2). WhileTable3 shows **Model II** which consist of all parameters in Model I + PON2 lactonase activity. (**R2 =0.209, p=0.001**) Area under ROC curve: 0.728 (Figure 3). Significant association between PON2 activity, and nitric oxide levels, and the risk of preeclampsia identified in univariate regression analysis remain significant after adjustment of other risk factors of preeclampsia, for PON2 (OR 2.071, p = 0.012) and for nitric oxide (OR 1.10, p = 0.025) (Table 3). This finding suggests that, PON2 activity is a predictor of LBW in preeclampsia and also along with NO and lipid profile it can predict the presence of low birth weight.

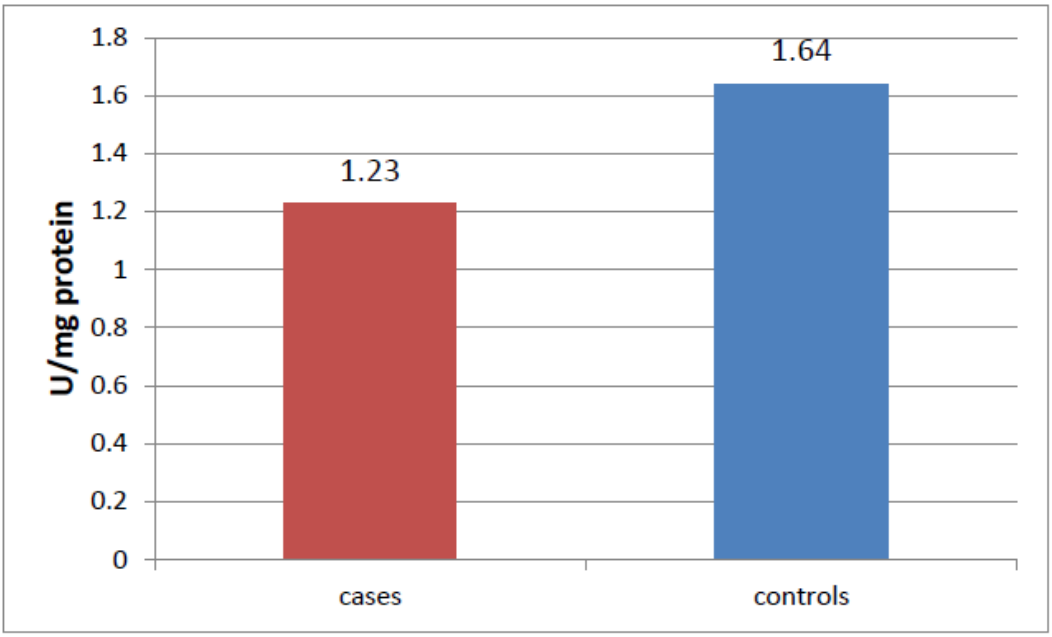

Figure 1

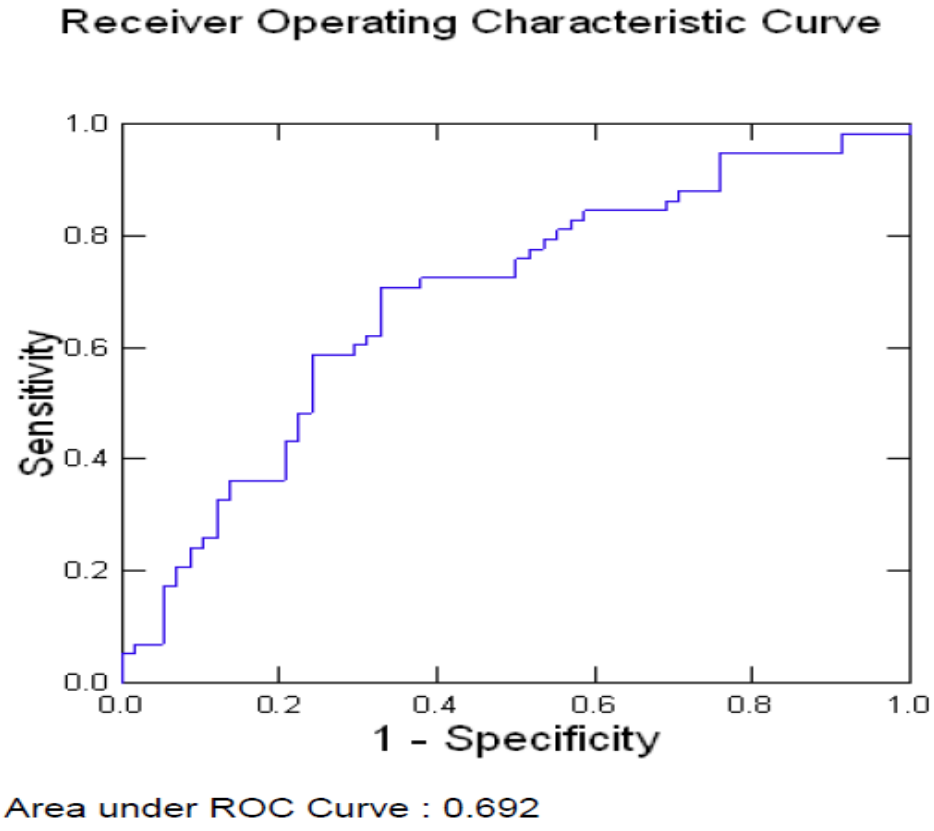

Figure 2

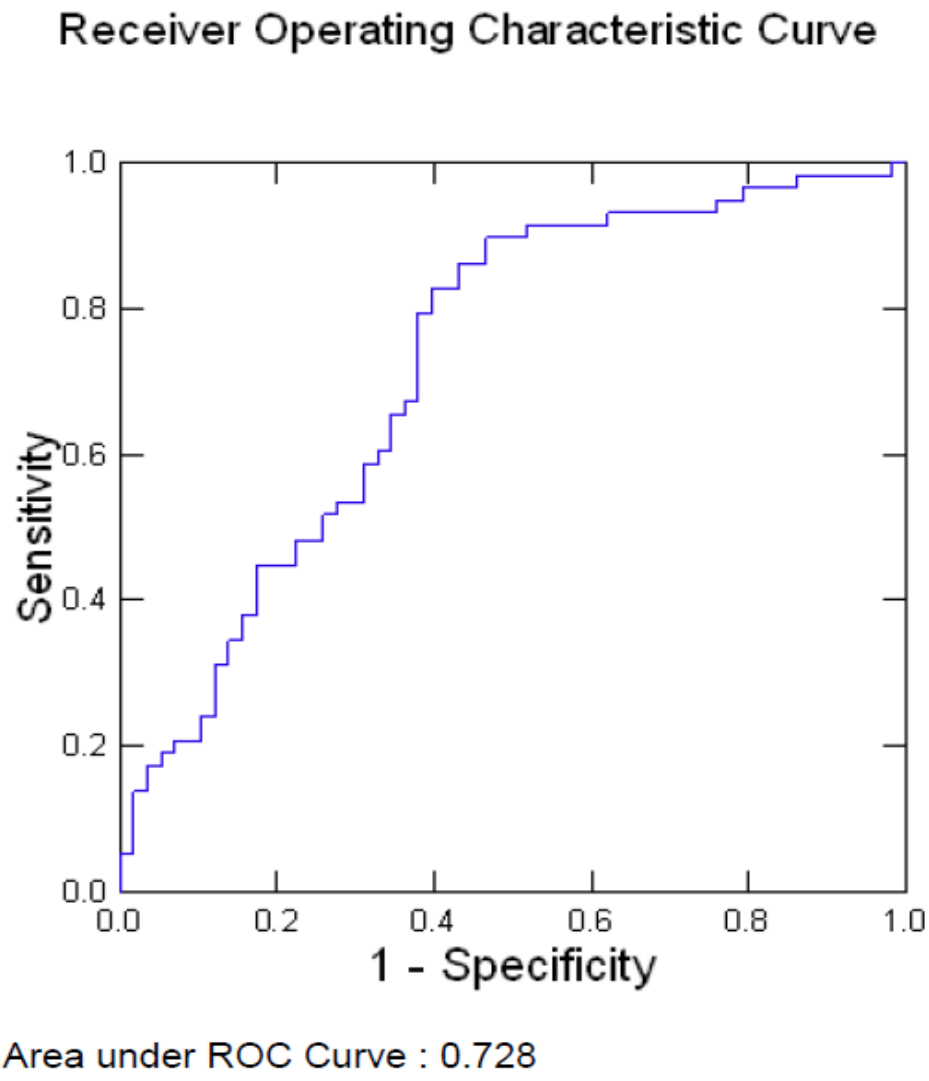

Figure 3

Table1. Biochemical parameters of pre-eclampsiacases and controls

Parameter	**Cases**	**Control**	**P value**
T.Cholesterol (mg/dl)	187.72±46.42	170.25±39.25	0.031
Triglyceride (mg/dl)	177.59±47.90	163.67±45.05	0.110
HDL-Cholesterol (mg/dl)	34.27±6.017	36.58±7.49	0.070
VLDL-Cholesterol (mg/dl)	35.46±9.51	32.88±9.19	0.139
LDL-Cholesterol (mg/dl)	114.17±32.08	98.24±31.91	0.008
Nitric oxide (umol/L)	22.96±4.83	24.96±4.99	0.031
PON2 (U/mg protein)	1.23± 0.81	1.64±0.69	0.003
Birth weight	2.52±0.62	2.92±0.42	< 0.001

Table2.•Model 1, Logistic Regression Analysis (Naglekerke R2=0.141, p= 0.012)

Independent Variables	**coefficient**	**Z value**	**SE**	**OR (95% CI)**	**P value**
Constant-	1.251	-0.751	1.664	-	0.452
Total Cholesterol	-0.001	-0.131	0.009	0.999(0.982-1.016)	0.896
HDL-Cholesterol	0.025	0.822	0.031	1.025(0.966-1.089)	0.411
LDL-Cholesterol	-0.014	-1.190	0.012	0.986(0.964-1.001)	0.234
Nitric Oxide	0.085	2.083	0.041	1.089(1.005-1.180)	**0.037**

Table 3.•Model 2,Logistic Regression Analysis (Naglekerke R2 =0.209, p= 0.001)

Independent variables	**Coefficient**	**Z value**	**SE**	**OR (95% CI)**	**Pvalue**
Constant	-3.185	-1.692	1.882	-	0.091
Total Cholesterol	-0.005	-0.520	0.010	0.994(0.975-1.012)	0.603
HDL-Cholesterol	0.035	1.082	0.032	1.036(0.972-1.103)	0.279
LDL-Cholesterol	-0.005	-0.375	0.013	0.995(0.971-1.020)	0.708
Nitric Oxide	0.096	2.421	0.043	1.110(1.012-1.196)	**0.025**
PON2	**0.728**	**2.514**	**0.290**	**2.071(1.174-3.653)**	**0.012**

Discussion:

Main findings:

In the present study Paraoxonase2 activity was significantly lower in preeclamptics compared with controls. Serum nitric oxide decreased significantly in cases. There is significant positive association is found in linear regression analysis between PON2 and Birth weight. PON2 lactonase activity was negatively correlated with serum cholesterol. Multivariate regression analysis shows that PON2 lactonase could add up the diagnostic predictability of preeclampsia.

Strength:

To the best of our knowledge, the present study is the first in which PON2 lactonase is done to assess correlation between birth weight of babies and found significant association with birth weight.

Interpretation:

Our results are in line with majority of previous studies in this field as study done by Belo *et al*, Hubel *et al*, who reported significant relationship between hyperlipidemia, and pre-eclampsia [10,11]. Enquobahrie 2004, demonstrated that early pregnancy dyslipidemia is associated with an increased risk of pre-eclampsia[12]. Comparing our results and those of other studies, the role of hypertriglyceridemia and high LDLc cholesterol level in pathogenesis of preeclampsia is confirmed in majority of studies.

De *et al* found that the elevated serum concentrations of TG in pre-eclampsia patients which are in good agreement with the results of our study. The elevated concentrations of serum TG in pre-eclampsia can be explained by higher levels of free fatty acid in conjunction with reduced hepatic β-oxidation [13]. There is conflicting

evidence about the serum cholesterol Winkler K *et al.* reported that serum cholesterol was significantly lower in preeclampsia [14]. Bayhan *et al.* found that there are elevated circulating levels of lipid peroxides in pre-eclampsia [15]. He also suggested that imbalance between lipid peroxidation and antioxidants were an important factor in the pathogenesis of pre-eclampsia.

These findings support the importance of the atherogenic lipid profile that is enhanced in pre-eclampsia which may be significant contributors to endothelial dysfunction. Christopher P observed genetic variation in PON2 genotype influencing the birth weight of patients but the activity was not performed [16]. Ng Carey *et al.* 2001 demonstrated that unlike PON1, which, PON2 is not found in the circulation and acts as an intracellular antioxidant suggest that PON2 possesses antioxidant properties similar to those of PON1 and PON3 [17]. Horke *et al*. suggest that PON2 represents an endogenous defence mechanism against vascular oxidative stress thereby contributing to the prevention of atherosclerosis [18]. A decreased PON2 expression has been observed in hypercholesterolemic patients and during progression of atherogenesis [19]. Altenhofer *et al.* recently shown that the human enzyme Paraoxonase-2 (PON2) has two functions an enzymatic lactonase activity and the reduction of intracellular oxidative stress. By its antioxidative effect, PON2 reduces cellular oxidative damage and influences redox signalling, which promotes cell survival [19] Thus, it is of interest to explore whether pregnancy complications such as pre-eclampsia add to the imbalance between PON2 as antioxidants in preeclamptics. Ng C J *et al.* recently shown that PON2 over expression in cells was shown to reduce intracellular oxidative state and the cells' ability to oxidize LDLc[20]. Fortunato *et al*. recently demonstrated that, in human macrophages, only PON2 (but not PON1 and PON3) is expressed, and its expression is increased under oxidative stress [21].

Our finding of negative correlation between PON2 and total cholesterol is supported by Resenblat *et al.* Who found human monocyte derived macrophages PON2 expression is reduced in patient with hypercholesterolemia as a result of their increased cellular cholesterol content [19]. Whereas in study conducted by Fortunato *et al.* an animal model showed PON2 protects against atherogenesis in vivo by modulating lipoprotein properties, thereby reducing cellular oxidative stress [20]. Pathophysiology of pre-eclampsia has shown association with atherogenic wall changes in the uteroplacental bed [22]. With the knowledge of this our results of decreased PON2 in pre-eclampsia can be explained as follows. Lowered PON2 is due to excess utilization by the inflamed tissues to scavenge the excessive lipid peroxides that are generated at inflammatory sites, or to scavenge accumulated lipid peroxides in plasma. The conclusions regarding the association between nitric oxide and pre-eclampsia are conflicting. Sandrim *et al* showed results in aggrement with our result i.e. decreased serum nitric oxide in preeclamptics compared to controls [23]. Davidge *et al* reported that urinary nitric oxide metabolites are decreased in preeclamptics [24]. Nitric oxide (NO) mediates many functions of the endothelium, including vasodilatation and inhibition of platelet aggregation. Di Iorio R *et al.* and Diejomaoh *et al* showed no significant change and decreased levels found in study by Seligman *et al* in their content [25-27]. PON2 may be inactivated by attack of hydroxyl radicals,

direct oxidation by peroxides [28]. Oxidative stress, ultimately affects the birth weight compromising nourishment of fetus. One possible explanation for our finding of decreased PON2 lactonase activity in low birth weight is the susceptibility of the PON2 to get inactivated by oxidative damage or increased consumption. The endoplasmic reticulum stress is one of the sources of reactive oxygen species (ROS) through protein misfolding [29]. Another explanation can be atherogenic changes in the placental circulation limiting blood flow to fetus. Such compromised blood flow results in tissue hypoxia that causes ROS other and producing nitric oxide (NO) stress, oxidative triggers [30]. Placental oxidative stress is reported to be involved in the etiopathogenesis of IUGR [31]. There is reduced trophoblastic invasion in IUGR and small for gestational age babies. This deficient spiral artery conversion predisposes to placental malperfusion due to lipid-laden mononuclear cells forming intimal plaques [32,33]. Such oxidative modifications of LDL in plaque can have its role in decreasing the PON2 lactonse activity [34]. Collectively, the data provide convincing evidence that oxidative stress and especially lipid peroxidation are abnormally increased in the placentas of pre eclamptic women several investigators studied the relationship between the oxidative state of the mother and the newborn at the moment of birth [35]. Auguelles S. et al. measured oxidative stress markers lipid peroxides and total antioxidant capacity (TAC) and found a good correlation between the oxidative status of the mother and of the neonate. Placental generation of ROS and reactive nitrogen species in preeclampsia might be facilitated by a reduction in local antioxidant defence [35]. PON2 the enzyme limits the accumulation of lipid peroxides. As increased cholesterol levels were shown in the cases we hypothesized that this phenomenon may also exist in the patients' monocyte, which are the hallmark of early atherogenesis. The patients' monocyte may get differentiated into macrophage foam cells. In conclusion in patients with hypercholesterolemia, reduced cellular PON2 expression might be the underlying contributors to their accelerated atherosclerotic changes in placenta leading to pre-eclampsia. In support of our observation one study showed increase HMDM-PON2 expression is reduced in patients with hypercholesterolemia as a result of their increased cellular cholesterol content [36].

These results indicate consumption of antioxidants to combat heightened lipid peroxidation, which may injure vascular endothelium, and likely be involved in the pathogenesis of preeclampsia.

Limitations:

Smaller sample size could be the limiting factor for our study.

Conclusion:

Our study shows that PON2 lactonase is reduced in preeclamptics and shows significant association with birth weight. Such reduction in activity may be related to oxidative stress through either ER stress or atherogenic changes in placental circulation through oxidative modifications in LDL. In future, further studies are needed in this direction to assess the effect of the oxidative stress on fetus through fetal PON2 in its long term health prospective.

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Consent:
All women gave informed written consent to participate in the study, which had been approved by the institutional Ethics Committee of S. R. T. R. Govt. Medical College. Ambajogai on 9/9/2010.

Conflict of interest:
The authors do not have any conflict of interest. This project was not been funded by any organisation.

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