ASSOCIATION OF HUMAN MONOCYTIC PARAOXONASE 2 (PON 2) AND SERUM NITRIC OXIDE LEVELS IN PREECLAMPSIA PATIENTS

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ABSTRACT: OBJECTIVE: Pre-eclampsia and atherosclerosis are both endothelial diseases with an involvement of lipid-mediated oxidative damage. The intracellular antioxidant enzyme paraoxonase 2 (PON2) may have a protective function in the prevention of atherogenesis. Oxidative stress have important role in the pathogenesis of pre-eclampsia. Study was designed to investigate monocytic paraoxonase 2 activity in pre-eclampsia. **DESIGN AND METHOD:** Study group was case-control study of 60 with preeclampsia and 60 uncomplicated term-deliveries. Paraoxonase2 activity done using substrate Dihydrocoumarin (DHC). **RESULTS:** Monocytic Paraoxonase2 activity was significantly lower in women with preeclampsia v/s controls (1.22U/mg versus 1.67U/mg protein, $p = 0.002$) Serum levels of T. chol, LDLc and VLDLc are higher in cases than in controls and are statistically significant. Serum nitric oxide also showed significant decrease in cases 22.92umol/L versus 25.12umol/L, $p = 0.015$. In multivariate regression analysis PON2 (OR 2.393, $p = 0.002$) and for nitric oxide (OR 1.091, $p = 0.030$). Multivariate logistic regression analysis after adjustment of other risk factors demonstrates decreased PON2 lactonase activity is associated with greatest risk for preeclampsia. Model 1:, total cholesterol, HDL-C, LDL-C, nitric oxide ($R^2 = 0.137$, $p = 0.011$), model 2 All parameters in Model 1 + PON2 lactonase activity. ($R^2 = 0.243$, $p < 0.001$). ROC plots demonstrated a high diagnostic accuracy for model 2 (area under ROC curve = 0.745) than model1 (area under ROC curve = 0.686) **CONCLUSION:** decreased PON2 lactonase activity, abnormal lipid profile and decreased nitric oxide (nitrate+ nitrite) may have role in pathogenesis of pre-eclampsia and also gives valuable information regarding the risk prediction of pre-eclampsia.

KEYWORDS: Preeclampsia, Paraoxonase2

INTRODUCTION: Pre-eclampsia is a multisystem disorder of unknown etiology characterized 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. From evolutionary stand point, PON2 appears to be the oldest member, followed by PON3 and PON1 ⁽³⁾ PON2 has lactonase activity ⁽⁴⁾. It is an intracellular protein which protects cells against oxidative damage. ⁽⁵⁾

PON2 expressed in cells of the artery wall including an endothelial cells, smooth muscle cells & macrophages, also predominantly expressed in monocytes and influence lipoprotein properties and cellular oxidation. Plasma lipids and monocytes are important component in blood which are contributing factor of the atherogenesis of blood vessels ⁽⁶⁾. Major contributors to atherosclerosis are oxidative damage and endoplasmic reticulum (ER) stress-induced apoptosis; both of which can be diminished by the anti-oxidative protein PON2 expressed in monocyte ⁽⁷⁾.

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Taking the deleterious effects of oxidized lipoproteins on endothelium and PON2 enzyme's protective effect on lipoprotein oxidation into consideration, study was designed to investigate serum PON2 activity and pre-eclampsia. Even modest increases in the simultaneous production of superoxide and NO will greatly stimulate the formation of peroxynitrite which damages the endothelium leading to preeclampsia⁽⁸⁾.

This evolutionary perspective raises the question of establishment of interlink between antioxidant protein PON2, and NO in pathophysiology of pre-eclampsia. Early identification of pregnant women at risk for pre-eclampsia is a priority to implement preventive measures. Some biochemical and ultra sonographic parameters have shown promising predictive performance, but so far there is no clinically validated screening procedure.

MATERIALS AND METHODS: This is a hospital based case control study. Total 120 pregnant females enrolled in this study. Sixty 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 60 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. 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, and connective tissue diseases acute or chronic hepatic diseases).

Fasting blood samples were obtained from ante cubital 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 milliliters of blood was transferred into heparinized 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 1500g for 10 minutes which was then used for estimation of nitric oxide and lipid parameters. Serum analytes were estimated by ERBA Smart lab auto analyzer. Analysis was performed within 24 hours kept in freezer compartment till analysis. All chemicals used were of reagent grade. Serum nitric oxide (nitrate + nitrite) estimation was done using method of Katrina Miranda⁽⁹⁾. Lysed monocyte protein estimation was assessed using Lowry's method.⁽¹⁰⁾ Lipid parameters were done using routine laboratory method.

MonocytePON2 Lactonase Activity done as described earlier⁽⁷⁾. The final results are expressed as U/mg of protein, after estimation of protein content (Lowry's method) in monocyte lysate.

The results obtained in the study were evaluated using MYSTAT STATISTICAL PACKAGE at 95% confidence interval and at a significance level of $p < 0.05$.

Results are presented as mean $\pm$ 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. The strength of association between two parameters is expressed by the Pearson's

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correlation coefficient. The logistic regression analysis is used for prediction of risk of pre-eclampsia contributed by various risk factors.

RESULTS: There were no differences in maternal characteristics between the two groups, with regard to age, number of pregnancies and delivery type. Mothers participating in the study were predominantly, 20–35 years old. There was no significant difference in mean age between women with pre-eclampsia (23.85 ± 3.38 years) and controls (22.75 ± 2.89 years). Serum levels of total cholesterol, Low density lipoprotein-cholesterol and very low density lipoprotein cholesterol were 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 also showed significant decrease in cases (22.92 ± 4.75 $\mu\text{mol/L}$) as compared to control group (25.12 ± 4.99 $\mu\text{mol/L}$), ($p = 0.015$).

Monocyte PON2 also showed significant decrease in cases (1.22 ± 0.80 U/mg protein) as compared to control group (1.67 ± 0.70 U/mg protein) ($p = 0.002$). (Table 1) (Fig. 1). Multivariate logistic regression analysis was 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 R^2 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 development of preeclampsia. Model 1 ($R^2 = 0.137$, $p = 0.011$), (Table. 2).

Model 2 consist of all parameters in Model 1 + PON2 lactonase activity. ($R^2 = 0.243$, $p < 0.001$), (Table. 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 (Odds Ratio [OR] 2.393, $p = 0.002$) and for nitric oxide (OR 1.091, $p = 0.030$). ROC plots demonstrated a high diagnostic accuracy for model 2 (area under ROC curve = 0.745) (Fig. 2) than model 1 (area under ROC curve = 0.686) (Fig. 3).

DISCUSSION: In this case control study, we tested the hypothesis that the relationship between PON2, lipid profile and nitric oxide with the pre-eclampsia differs from normal pregnancy. To the best of our knowledge, the present study is the first that makes use of all these markers together for the systematic analyses of women with pre-eclampsia.

Strengths of this study are to bring light on proteins like PON2 that were not previously thought to be modified in pre-eclampsia and provide an independent confirmation that several already identified factors are modified in pathological pregnancies.

There are several potential limitations to the present study. The study is conducted on small sample size as pre epidemiological point. Such small sample size may not offer sufficient power to interpret the results of serum parameters. One more limitation is we have not done the polymorphism of PON1 which influences the PON1 activity. Further studies are needed to characterize the molecular mechanism by which PON2 involved in preventive process of pre-eclampsia.

In the present study, the levels of serum cholesterol and LDLc were higher and high-density lipoprotein was significantly lower in pre-eclampsia patients compared with that of the control

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women. The levels of TG and VLDLc are also found to be increased in pre-eclampsia though not statistically significant. Our results are in line with majority of previous studies in this field who reported significant relationship between hyperlipidemia, and pre-eclampsia, especially for triglyceride^(11, 12).

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, enhanced peripheral insulin resistance, and reduced catabolism of TG.⁽¹³⁾ There is conflicting evidence about the serum cholesterol one study reported that serum cholesterol was significantly lower in preeclampsia⁽¹⁴⁾.

Our finding that HDLc is decreased significantly in pre eclampsia is in agreement with various studies^(11, 16, 17). HDL-cholesterol level was reported as unchanged in one study⁽¹²⁾. Previous found that there are elevated circulating levels of lipid peroxides in pre-eclampsia this suggested that imbalance between lipid peroxidation products and antioxidant activity was an important factor in the pathogenesis of pre-eclampsia.⁽¹⁵⁾ Several in vivo and in vitro studies support this notion. These findings support the importance of the atherogenic lipid profile that is enhanced in pre-eclampsia, and these findings may be significant contributors to endothelial dysfunction.⁽¹⁷⁾

Previous study 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⁽¹⁸⁾.

However, its antioxidant functions at the cellular level, joining the host of intracellular antioxidant enzymes that protect cells from oxidative stress⁽¹⁹⁾. PON2 represents an endogenous defense mechanism against vascular oxidative stress and unfolded protein response-induced cell death, thereby contributing to the prevention of atherosclerosis⁽²⁰⁾. A decreased PON2 expression has been observed in hyper cholesterolemic patients and during progression of atherogenesis⁽²¹⁾.

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 signaling, which promotes cell survival⁽²⁰⁾. Thus, it is of interest to explore whether pregnancy complications such as pre-eclampsia add to the imbalance between PON2 as antioxidants in pre eclampsia. This study provided the first comparison of antioxidant status i.e. PON2 among preeclampsia and normal pregnancy. It has been recently shown that PON2 over expression in cells was shown to reduce intracellular oxidative state and the cells' ability to oxidize LDLc⁽¹⁸⁾.

In human macrophages, only PON2 (but not PON1 and PON3) is expressed, and its expression is increased under oxidative stress⁽²²⁾. Emerging data in the human and animal literature suggests that protective effect of PON2 could fail during atherosclerosis exacerbation and human monocyte derived macrophages PON2 expression is reduced in patient with hypercholesterolemia as a result of their increased cellular cholesterol content,⁽²³⁾ whereas in one animal model showed PON2 protects against atherogenesis in vivo by modulating lipoprotein properties, thereby reducing cellular oxidative stress and attenuating the inflammatory response.⁽²²⁾

Pathophysiology of pre-eclampsia has shown association with atherogenic wall changes in the uteroplacental bed⁽²⁴⁾. With the knowledge of this our result 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. As PON2 is involved in protection against the oxidative stress

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one possible explanation for our finding of decreased PON2 lactonase activity in pre eclamptics is the susceptibility of the PON2 to get inactivated by oxidative damage or increased consumption.

Another possible explanation is increased generation of ROS leads to increased lipid peroxidation. This deficient spiral artery conversion predisposes to placental mal perfusion due to lipid-laden mononuclear cells forming intimal plaques. Such oxidative modifications of LDL in plaque can have its role in decreasing the PON2 lactonase activity. The conclusions regarding the association between nitric oxide and pre-eclampsia are conflicting decreased levels found in various studies are in agreement with our result i.e. decreased serum nitric oxide in pre eclamptics compared to controls ⁽²⁵⁾. Whereas, in one study urinary nitric oxide metabolites were decreased in pre eclamptics ⁽²⁶⁾. Nitric oxide (NO) mediates many functions of the endothelium, including vasodilatation and inhibition of platelet aggregation ⁽²⁷⁾.

CONCLUSION: Finally we conclude that the results obtained in our study suggest decreased PON2 lactonase activity, abnormal lipid profile and decreased nitric oxide (nitrate + nitrite) may have a role in pathogenesis of pre-eclampsia and also give valuable information regarding the risk prediction of pre-eclampsia. 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.

The authors do not have any conflict of interest. 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. This project was not been funded by any organisation. Dr S. D. Sawant had carried out all the process of analysis of samples had also done the statistical analysis and writing up of the work. Dr M. R. Mogarekar had role in the conception and planning of this project.

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Parameter	Cases	Control	P Value
Age (years)	23.85±3	38 22.75±2.89	0.058
T. Cholesterol (mg/dl)	185.55±47.22	169.75±38.72	0.047
Triglyceride (mg/dl)	177.53±47.57	162.30±44.92	0.074
HDL-Cholesterol (mg/dl)	34.10±5.99	36.63±7.41	0.042
VLDL-Cholesterol (mg/dl)	35.45±9.43	32.58±9.18	0.094
LDL-Cholesterol (mg/dl)	112.32±33.13	98.07±31.39	0.017
Nitric oxide (umol/L)	22.92±4.75	25.12±4.99	0.015
PON2 (U/mg protein)	1.22± 0.80	1.67±0.70	0.002

Table1. Biochemical parameters of pre-eclampsia cases and controls

Independent Variables	Coefficient	Z value	SE	OR (95% CI)	P value
Constant	-1,788	-1,111	1.608	-	0.266
Total Cholesterol	-0.001	-0.167	0.999	0.999(0.982-1.016)	0.867
HDL-Cholesterol	0.031	1.020	0.031	1.032(0.972-1.096)	0.308
LDL-Cholesterol	-0.011	-0.971	0.011	0.989(0.967-1.011)	0.332
Nitric Oxide	0.088	2.164	0.041	1.092(1.008-1.183)	0.030

Table 2: Model 1, Logistic Regression Analysis (Naglekerke R²=0.137, p= 0.011)

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Independent Variables	Coefficient	Z value	SE	OR (95% CI)	P value
Constant	-2.804	-1.637	1.713	-	0.102
Total Cholesterol	-0.006	-0.678	0.010	0.994(0.975-1.012)	0.498
HDL-Cholesterol	0.025	0.761	0.033	1.025(0.962-1.093)	0.447
LDL-Cholesterol	-0.007	0.530	0.012	0.994(0.970-1.018)	0.596
Nitric Oxide	0.104	2.421	0.043	1.110(1.020-1.208)	0.015
PON2	0.873	3.163	0.276	2.393(1.394-4.109)	0.002

Table 3: Model 1, Logistic Regression Analysis (Naglekerke R² =0.243 , p= 0.000)

Fig. 1

Fig. 2

Area under ROC Curve: 0.745

Fig. 3

Area under ROC Curve: 0.686

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