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## **Role of Pro-oxidant Myeloperoxidase and an Oxidative Stress Marker Malondialdehyde in Prediction of Preeclampsia**

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### **Authors' contributions**

*This work was carried out in collaboration between all authors. Author SMRU designed the study. Author RR wrote the protocol. Authors SMRU, BMR and HVS supervised the work. Author RR carried out all laboratories work and performed the statistical analysis. Authors RR and SMRU managed the analyses of the study. Author RR wrote the first draft of the manuscript. Authors SMRU and RR managed the literature searches. Authors SMRU, BMR and HVS edited the manuscript. All authors read and approved the final manuscript.*

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### **ABSTRACT**

**Aim of the Study:** The treatment of preeclampsia (PE) is symptomatic till date, as the etiology of this condition has remained elusive for many centuries. Appreciating polymorphonuclear activation and oxidative stress as the early events in the pathogenesis of preeclampsia, we hypothesized that the activity of Myeloperoxidase (MPO), a pro-oxidant enzyme and Malondialdehyde (MDA), an oxidative stress marker may be elevated in preeclamptic subjects. Hence, we set out to estimate their levels in a representative sample of south Indian women with preeclampsia and compare them with controls (uncomplicated, normotensive pregnant women of more than 20 weeks of gestation).

**Study Design:** Cross sectional study.

**Place and Duration of Study:** Pregnant women attending the Antenatal clinic of Rajarajeswari

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Medical College and Hospital, Bengaluru, from May 2012 to May 2013 were our study subjects.

**Materials and Methods:** Fifty uncomplicated, normotensive pregnant women of more than 20 weeks of gestation and fifty clinically diagnosed cases of preeclampsia were our controls and cases respectively. Pregnant women with medical conditions that interfere with the study were excluded. Serum MPO activity and MDA levels were estimated manually by spectrophotometric method.

**Results:** There was significant ( $P < 0.001$ ) increase in MPO levels and MDA levels in preeclampsia patients compared to controls.

**Conclusion:** There was increase in pro-oxidant and oxidative stress marker levels indicating an oxidative stress in PE. Thus this study shows that oxidative stress may play a key role in the pathogenesis of PE which contributes to endothelial dysfunction and maternal signs and symptoms. Estimation of MPO and MDA in early pregnancy may help in identifying pregnant women at risk of developing preeclampsia and execution of preventive measures.

*Keywords: PE; MPO; MDA; oxidative stress.*

## 1. INTRODUCTION

Preeclampsia (PE) is a pregnancy specific syndrome that can affect virtually every organ system. It is a disorder of widespread vascular endothelial malfunction and vasospasm that occurs after 20 weeks of gestation, clinically characterized by new onset of hypertension, proteinuria with or without edema. The global incidence of preeclampsia has been estimated to be 5%-14% of all pregnancies. It is the third leading pregnancy related cause of maternal death, after hemorrhage and embolism (790 maternal deaths per 100,000 live births) and 15% of preterm births [1,2,3].

The treatment of PE is symptomatic till date, as the etiopathogenesis is not yet fully understood; numerous theories have been put forward emphasizing placental oxidative stress as the major mechanism involved in the pathogenesis of preeclampsia. Abnormal placentation in preeclampsia leads to placental hypoxia which in turn initiates a cascade of secondary effector mechanisms causing imbalance between pro-oxidants (reactive oxygen species) and antioxidants leading to maternal oxidative stress, immunological dysfunction and endothelial damage resulting in clinical signs and symptoms of preeclampsia [1,2,3].

Myeloperoxidase (MPO EC 1.11.1.7) a leukocyte derived pro-oxidant enzyme may contribute to the oxidative damage in the endothelium and placenta of women with preeclampsia. Traditionally, MPO dependent mechanism is the most potent oxygen dependent bactericidal system in neutrophils and monocytes. Recent investigations have suggested that MPO is secreted also from certain tissue macrophages (such as in atherosclerotic plaques) and plays a

role in several inflammatory conditions and in atherosclerotic process, which can lead to several life-threatening conditions [4]. Accumulating evidence also suggests that preeclampsia (PE) and cardio vascular diseases share similar disturbances like enhanced systemic inflammatory response and endothelial dysfunction. MPO activity has been extensively studied in cardiovascular diseases and their elevated levels have been implicated as an independent marker of coronary artery disease. [4,5]. On the contrary, to the best of our knowledge, very few researchers have explored the role of MPO in preeclampsia in Indian women. This observation prompted us to undertake this study.

Uncontrolled lipid peroxidation, in view of its potentially destructive character has been suggested as an etiological factor in preeclampsia [6]. In addition to MPO, we have also studied serum Malondialdehyde (MDA) levels- a widely used indicator of lipid peroxidation.

The aim of the study is to assess oxidative stress by estimating serum Myeloperoxidase (MPO) activity and Malondialdehyde (MDA) levels in preeclampsia (cases) and in controls (uncomplicated, normotensive pregnant women of more than 20 weeks of gestation) and to compare both the groups.

## 2. MATERIALS AND METHODS

Ethical clearance was obtained from Institutional ethical clearance committee. After obtaining informed consent and under full aseptic precautions, from both the study groups, 5 ml of venous blood sample was collected in vacutainers containing clot activators. Fifty

pregnant women of more than 20 weeks of gestation both primigravida and multigravida with no bad obstetric history and fifty pregnant women who had been clinically diagnosed with preeclampsia (both primigravida and multigravida) attending Antenatal Clinic of RRMCH, Bengaluru were our study controls and cases respectively.

Pregnant women with h/o smoking, alcoholism, gestational diabetes, diabetes mellitus, hypertension, cardiovascular disease, chronic liver and kidney diseases, anemia, multiple pregnancies and other chronic diseases that interfere with the study, pregnant women on antioxidants like Vitamin E and Vitamin C supplementation were excluded from the study.

Serum MPO and MDA levels were estimated spectrophotometrically (ELICO company, SL-159 Single Beam Microprocessor based Scanning UV-Visible Spectrophotometer). Serum MPO levels were estimated using ortho Dianisidine dihydrochloride (Sigma-Aldrich Company) as a substrate. This method is based on the oxidation of o-Dianisidine (colorless chromogen) by hydrogen peroxide, to form an yellowish orange colored oxidized product in presence of MPO. 0.1 ml of fresh serum sample and 2.9 ml of 50 mM Phosphate buffer at pH 6.0 were incubated in water bath at 25°C for 10 min and then 0.1 ml of freshly prepared o-Dianisidine dihydrochloride was added. The above mixture was mixed well and then 0.1 ml of 1% H<sub>2</sub>O<sub>2</sub> solution was added. The absorbance was measured at 470 nm for 3 min continuously (kinetic assay). The color of the solution slowly turns yellow and then to orange. A reagent blank was run along with this using 0.1 ml of distil water instead of serum sample. One unit (U) of (MPO) activity was defined as that degrading one micromole of peroxide per minute at 25°C [7]. The lower limit of detection of the assay method was 10 U/L. Intra and inter assay variation was 8% and 10% respectively.

Serum MDA was estimated by thiobarbituric acid reactive substances (TBARS) method described by Wilbur et al. [8,9]. Fresh serum sample was heated with thiobarbituric acid (TBA) (from Hi Media Company) under acidic conditions and the amount of pink colored MDA-TBA adduct produced was measured colorimetrically at 530 nm in spectrophotometer. 0.5 ml of fresh serum was pipetted into 'T' test tube and 0.5 ml of distilled water into test tube 'B'. Then 0.5 ml of 40% TCA and 1 ml of 0.67% freshly prepared TBA was added to each. The contents in the tube were mixed and then placed in boiling water bath for ten minutes. Then the tubes were removed and cooled under ice cold water. Then mixture was centrifuged at 6000 rpm for 15 minutes. Absorbance of supernatant was read at 530 nm. MDA content of sample was calculated using molar extinction coefficient 1.56 X 100000 and expressed as nmol/ml of serum.

## 2.1 Statistical Analysis

The data was analyzed using statistical software namely SAS 9.2 and SPSS 15.0. Student't' test has been used to find the significance of study parameters. P value of less than 0.01 was considered as statistically significant. Microsoft word and Excel have been used to generate graphs and tables.

## 3. RESULTS

It is a cross sectional study. In our study 78% of the cases and 64% of controls were primigravida and 22% of cases and 36% of controls were multigravida. Mean age of the cases was 25±4 years and controls was 23±3 years. Mean gestational age of cases was 33.78±3.7 weeks and controls was 34.56±2.76 weeks. There was significant difference in the systolic ( $P<0.001$ ) and diastolic ( $P<0.001$ ) blood pressure among cases and controls. We found significant difference ( $P<0.001$ ) in MPO and MDA levels between cases and controls.

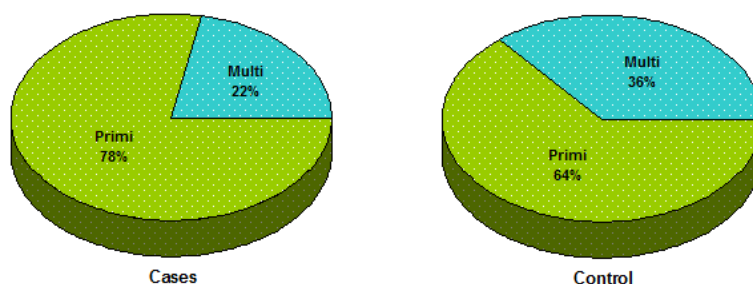


Fig. 1. Gravidity distribution of study groups

**Table 1. Age distribution of study groups**

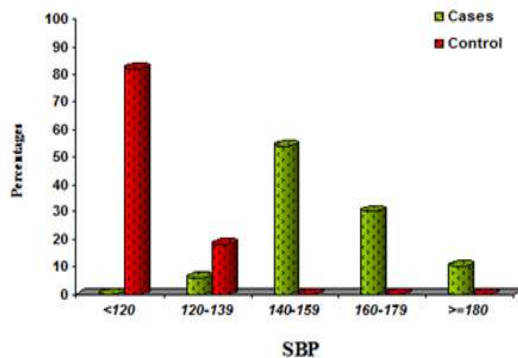
Age in years	Cases (pregnant women with preeclampsia)		Control (uncomplicated, normotensive pregnant women of > 20 weeks gestation)	
	No	%	No	%
18-20	5	10.0	16	32.0
21-25	25	50.0	27	54.0
26-30	16	32.0	7	14.0
31-35	4	8.0	0	0.0
Total	50	100.0	50	100.0
Mean ± SD	25.50±3.99		22.62±2.81	

**Table 2. Gestational age distribution of study groups**

Gestational age weeks)	Cases		Control	
	No	%	No	%
24-30	8	16.0	4	8.0
31-35	16	32.0	15	30.0
36-40	26	52.0	31	62.0
Total	50	100.0	50	100.0
Mean ± SD	33.78±3.70		34.56±2.76	

**Table 3. Range of study variables MPO and MDA among study groups**

Study variables	Cases	Control	P value
MPO (U/L)	52.79±12.57	22.91±9.53	<0.001**
MDA (nmol/ml)	8.33±2.16	3.98±1.13	<0.001**



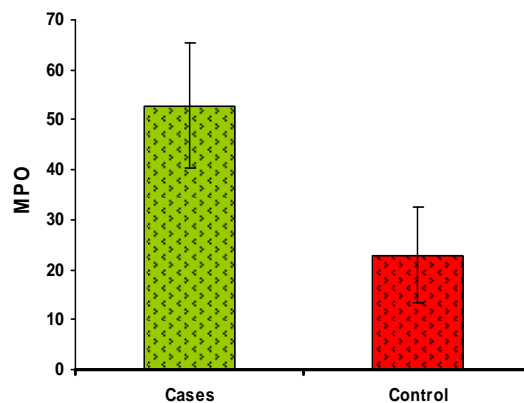
**Fig. 2. Comparison of blood pressure in study groups**

**4. DISCUSSION**

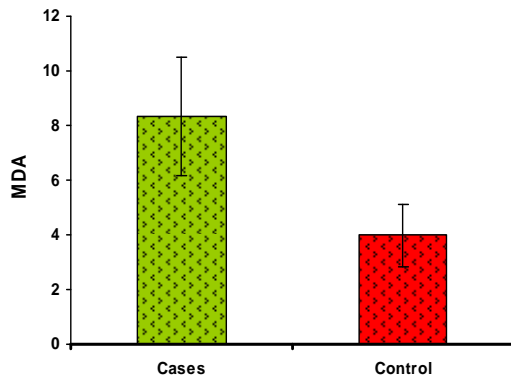
Preeclampsia is the commonest disorder of pregnancy with a multifactorial etiology and is called as 'disease of theories'. Increasing clinical and biochemical evidences suggest that disturbance in the normal endothelial cell function induced by oxidative stress may be a primary cause in the etiopathogenesis of preeclampsia. In this regard several authors have studied oxidative stress in preeclampsia by estimating various oxidants and antioxidants. In

the present study, we have evaluated the oxidative stress by estimating the activities of serum MPO and MDA levels in preeclampsia and compared with controls.

Both cases and controls in our study were age and gestationally age matched. Majority of the study subjects were primigravida. Among 50 cases, 86% (n=43) of the preeclamptic women were treated with anti-hypertensive drugs.



**Fig. 3. Comparison of MPO activity among study groups**



**Fig. 4. Comparison of MDA levels among study groups**

Myeloperoxidase is a hemoprotein, produced and released by activated neutrophils and monocytes. Serum levels of MPO reflect the MPO levels of activated neutrophils, monocytes and macrophages [10] and it is much easier and convenient to measure MPO levels in serum. MPO has plausibly been proposed to contribute to several phases of atherothrombosis seen in preeclampsia, from the initial insult to the vascular endothelium, to the development of atheroma, rupture of vulnerable atherosclerotic plaque and its clinical manifestations. MPO being a versatile enzyme reacts with several substrates in the body and releases several oxidant products that are able to react with structural and functional molecules in the host causing tissue damage. The oxidation products of MPO like oxidized LDL, oxidized apolipoprotein (apo) A1, HDL are responsible for the progression of inflammation and initiation of atherosclerosis. MPO may also have destabilizing effects on atheromatous plaque through the activation of metalloproteinases. MPO is also capable of using nitric oxide (NO) an endothelium-derived relaxing factor as a substrate to catalyze tyrosine nitration in proteins thereby consuming nitric oxide, contributing to endothelial dysfunction which is central to the etiology of preeclampsia [4].

In our study, serum MPO activity in preeclamptic women was significantly ( $P<0.001$ ) increased compared to controls. Robin E. Gandley et al. [4] and others [10,11] also have found increased activity of MPO in preeclamptic women compared to controls. Contrary to our finding Stepan H, et al. [12], in their study found no significant difference between plasma MPO concentration of pregnant women with normal

perfusion and the group with pathological uterine perfusion. Similar findings were also observed by Noyan et al. [13]. We also observed that serum MPO activity in treated (anti-hypertensive drugs) and untreated preeclamptic women did not differ significantly. Our observations were in agreement with Zhang et al. [14], who have reported that medications like anti-hypertensive drugs and lipid lowering agents did not appear to alter the MPO levels in patients with coronary artery disease. Interestingly about 30% of the PE women in our study, were found to have normal MPO activity similar to controls. The reasons may be multifactorial- ranging from environmental, immunological factors to nutritional status of the pregnant women. In addition, MPO gene polymorphism (e.g. -463G/A polymorphism of leukocyte MPO) could be an intriguing susceptibility factor that modulates an individual's risk of preeclampsia [15].

MDA is a highly reactive three carbon dialdehyde produced as a by-product of lipid peroxidation. It causes damage to LDL molecules, a crucial step in the pathogenesis of atherosclerosis. Lipid peroxidation products may also inhibit prostacyclin synthesis and stimulate smooth muscle contraction resulting in widespread vasospasm, a prominent feature of preeclampsia.

In our study we observed significant ( $P<0.001$ ) increase in MDA levels in cases compared to controls. Similar results were observed in several studies done in preeclampsia [16-18]. We did not find any significant difference in the MDA level among treated and untreated cases. Though it has been widely accepted as oxidative stress marker, Hackett C, et al. [19] differ in their observation and they have termed MDA to be a poor measure of in vivo lipid peroxidation. Maryam K.J. et al. [20] in their review article stress the need for re-evaluation of MDA as an oxidative stress biomarker. Despite substantial advances in understanding of PE, the development of simple tests to identify individuals or populations at risk still remains an epidemiologic and clinical challenge.

## 5. CONCLUSION

Preeclampsia is a significant obstetric complication in a developing country like India. This study, so far is one of the very few studies, to determine MPO activity in a representative sample of Indian women with preeclampsia. The

results of this study suggest that there is oxidative stress in preeclampsia which might cause endothelial damage and signs and symptoms of PE. Estimation of oxidative stress markers like MPO and MDA in the early trimester itself may help in the identifying pregnant women at risk of developing preeclampsia and execution of preventive measures. However the predictive value of MPO in preeclampsia in south Indian women has to be further evaluated in large scale prospective studies.

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### COMPETING INTERESTS

Authors have declared that no competing interests exist.

### REFERENCES

1. Shah MR. Hypertensive disorders in pregnancy. Etiopathogenesis of pre-eclampsia: Current concept and the controversies. JP Brothers. 1<sup>st</sup> Edition; 2007.
2. Hubel CA. Oxidative stress in the pathogenesis of preeclampsia. *Experimental Biology and Medicine*. 1999; 222(3):222-235.
3. George EM, Granger JP. Recent insights into the pathophysiology of preeclampsia. *NIH Public Access*. 2010;5(5):557-566.
4. Robin E. Gandley, et al. Increased myeloperoxidase in the placenta and circulation of women with preeclampsia. *NIH Public Access-hypertension*. 2008; 52(2):387-393.
5. Loria V, Dato I, Graziani F, Luigi MB. Myeloperoxidase: A New Biomarker of Inflammation in Ischemic Heart Disease and Acute Coronary Syndromes; 2008. Article ID 135625.
6. Kashinakunti SV, et al. Lipid peroxidation and antioxidant status in preeclampsia. *Al Ame en J Med Sci*. 2010;3(1):38-41.
7. Peter P, et al. Measurement of cutaneous inflammation: Estimation of neutrophil content with an Enzyme marker. *The Journal of Investigative Dermatology*. 1982;78:206-209.
8. Ramchandra KP, Ashok VS, Sangita MP. Lipid profile, serum malondialdehyde, superoxide dismutase in chronic kidney diseases and type 2 diabetes mellitus. *Biomedical Research*. 2012;23(2):207-210.
9. Wilbur KM, Bernheim F, Shapiro OW. The thiobarbituric acid method for malondialdehyde estimation. *Arch Biochem Biophysics*. 1943;250:305-313.
10. Mellembakken JR, Hogasen K, Mollnes TE, Hack CE, Abyholm T, Videm V. Increased systemic activation of neutrophils but not complement in preeclampsia. *Obstet Gynecol*. 2001; 97(3):371-4.
11. Hung TH, Chen SF, Lo LM, Li MJ, Yeh YL, Hsieh TT. Myeloperoxidase in the plasma and placenta of normal pregnant women and women with pregnancies complicated by preeclampsia and intrauterine growth restriction. *Placenta*. 2012;33(4):294-303.
12. Stepan H, Heihoff Klose A, Faber R. Pathological uterine perfusion in the second trimester is not associated with neutrophil activation. *Hypertens Pregnancy*. 2003;22(3):239-45.
13. Noyan T, Güler A, Sekeroğlu MR, Kamaci M, Aust NZJ. Serum advanced oxidation protein products, myeloperoxidase and ascorbic acid in pre-eclampsia and eclampsia. *Obstet Gynaecol*. 2006; 46(6):486-91.
14. Zhang R, et al. Association between MPO levels and risk of coronary artery disease. *JAMA*. 2001;286(17):2136-2142.
15. Ebru Ozturk, Ozcan Balat, Sacide Pehlivanab, Mete Gurol Ugur, Tugce Sever. Genetic variation of myeloperoxidase gene contributes to preeclampsia: A preliminary association study in Turkish population. *Hypertension in Pregnancy*. 2011;30(4):377-383.
16. Sharma JB, et al. Oxidative stress markers and antioxidant levels in normal pregnancy and pre-eclampsia. *Int J Gynaecol Obstet*. 2006;94(1):23-7.
17. Ayse Nur Aksoy, Nurinnisa Ozturk, Hulya Aksoy, Faith Akcay. Paraoxonase and arylesterase activities in patients with preeclampsia. *Eurasian J Med*. 2008; 40(1):10-13.
18. Meera KS, Maitra S, Hemalatha R. Increased level of lipid peroxidation in preeclamptic pregnancy; a relationship with paraoxanase 1 (PON1) activity.

- Indmedica-Biomedical Research. 2010; 20. Maryam Khoubnasabjafari, Khalil Ansarin, 21(4):10-12. Abolghasem Jouyban. Variations of MDA in PE. Hypertension in Pregnancy; 2016. Available:<http://dx.doi.org/10.3109/10641955.2016.1148163>
19. Hackett C, Linley-Adams M, Lloyd B, Walker V. Plasma malondialdehyde: A poor measure of *in vivo* lipid peroxidation. Clin Chem. 1988;34:208.

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