

Original Research Article

“STUDY OF SERUM MYELOPEROXIDASE ACTIVITY AND MALONDIALDEHYDE LEVELS IN PREECLAMPSIA”

Aim: The treatment of preeclampsia (PE) is symptomatic till date, as the etiology of this condition has remained elusive for many centuries. Oxidative stress may be the point at which multiple factors converge resulting in endothelial cell dysfunction and consequent clinical manifestations of preeclampsia. The full elucidation of the mechanisms involved in the pathogenesis will hopefully lead to a more complete understanding of the etiology of preeclampsia and lead to successful therapeutic intervention. The aim of the study is to assess the oxidative stress in preeclampsia by estimating the levels of Myeloperoxidase (MPO) a pro-oxidant enzyme and Malondialdehyde (MDA) an oxidative stress marker in preeclampsia and comparing them in normal pregnant woman.

Study design: Cross sectional study

Place and Duration of study: Pregnant women attending the Antenatal clinic of Rajarajeswari Medical College and Hospital, Bengaluru, between may 2012 to may 2013 were our study subjects.

Materials and Methods: Fifty PE women and fifty age matched normal pregnant women were cases and controls Pregnant women with medical conditions that interfere with the study were excluded. Serum MPO and MDA was 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 level indicating an oxidative stress in PE. Thus this study shows that oxidative stress plays 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 woman at risk of developing PE and execution of preventive measures.

Keywords: PE; MPO; MDA; Oxidative stress

25 **Introduction:**

26 Preeclampsia (PE) is a pregnancy specific syndrome that can affect virtually every organ system.
 27 It is a disorder of widespread vascular endothelial malfunction and vasospasm that occurs after 20
 28 weeks of gestation, clinically characterized by new onset of hypertension, proteinuria with or without
 29 edema¹. The global incidence of PE has been estimated to be 5%-14% of all pregnancies. It is the third
 30 leading pregnancy related cause of maternal death, after hemorrhage and embolism (790 maternal
 31 deaths per 100,000 live births) and 15% of preterm births^(1,2,3).

32 The treatment of PE is symptomatic till date, as the etiopathogenesis is not yet fully
 33 understood; numerous theories have been put forward. Epidemiological, clinical and molecular studies
 34 support that placental oxidative stress is the major mechanism involved in the etiopathogenesis of PE.
 35 Abnormal placentation in PE leads to placental hypoxia which in turn initiates a cascade of secondary
 36 effector mechanisms including altered proangiogenic and antiangiogenic factor balance, increase in
 37 maternal oxidative stress and endothelial and immunological dysfunction^(4,5,6).

38 Imbalance between pro-oxidants (reactive oxygen species) and antioxidants results in oxidative stress.
 39 Reactive oxygen species cause oxidation of unsaturated fatty acids and increases the lipid peroxidation
 40 products which may cause peroxidative damage to the vascular endothelium resulting in clinical
 41 symptoms of PE.

42 Myeloperoxidase (MPO EC 1.11.1.7) a pro-oxidant enzyme may contribute to the oxidative
 43 damage in the endothelium and placenta of women with PE. It is a leukocyte-derived enzyme stored
 44 abundantly in the azurophilic granules of monocytes and neutrophils, released in response to leukocyte
 45 activation for phagocytosis. The MPO dependent mechanism is most potent oxygen dependent
 46 bactericidal system in neutrophils and monocytes. Recent investigations have suggested that MPO is
 47 secreted also from certain tissue macrophages (such as in atherosclerotic plaques), mediate oxidation

of lipoproteins, causes depletion of endothelial derived nitric oxide and activation of metalloproteinases; thus also plays a role in several inflammatory conditions and in the atherosclerotic process, which can lead to several life-threatening conditions^(7,8,9).

Malondialdehyde (MDA) is a highly reactive three carbon dialdehyde produced as a by product of lipid peroxidation. MDA causes oxidation of 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 PE. Uncontrolled lipid peroxidation in view of its potentially destructive character has been suggested as an etiological factor in PE. Therefore, measurement of MDA is widely used as an indicator of lipid peroxidation⁽¹⁰⁾.

Aim of the study was to assess the oxidative stress by estimating Myeloperoxidase and Malondialdehyde in preeclampsia patients and to compare them with the activities in healthy pregnant women.

Materials and Methods:

Ethical clearance was obtained from Institutional ethical clearance committee. Blood samples were collected from both study and control group under full aseptic precautions after obtaining informed consent. Fifty pregnant women who have been clinically diagnosed with PE (both primigravida and multigravida) were cases and fifty age matched normal pregnant women of more than 20 weeks of gestation both primigravida and multigravida with no bad obstetric history, attending ANC of RRMCH, Bengaluru were our study controls.

Pregnant women with h/o smoking, alcoholism and with other conditions like gestational diabetes, diabetes mellitus, hypertension, cardiovascular disease, chronic liver and kidney diseases, anemia, multiple pregnancies and other chronic diseases that interfere with the study were excluded from the

study. Pregnant women on antioxidants like Vitamin E and Vitamin C supplementation were also excluded from the study.

Serum MPO and MDA levels were estimated spectrophotometrically. Serum MPO levels was estimated using O-Dianisidine dihydrochloride (HI Media Company) as a substrate¹⁰. This method is based on the oxidation of O-Dianisidine (colorless chromogen) by hydrogen peroxide to form a yellowish orange colored oxidized product in presence of MPO. 0.1ml of serum sample and 2.9ml of 50mM Phosphate buffer at pH 6.0, incubated in water bath at 25°C for 10min and then 0.1ml of O-Dianisidine Dihydrochloride added. Above mixture was mixed well and then 0.1ml of 1% H₂O₂ solution was added. The absorbance was measured at 470nm 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 0.1ml 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⁽¹¹⁾.

Serum MDA was estimated by thiobarbituric acid reactive substances (TBARS) method described by Wilbur et al^(12,13). Serum sample was heated with thiobarbituric acid (TBA) under acidic conditions and the amount of pink colored MDA-TBA adduct produced was measured colorimetrically at 530nm in spectrophotometer. 0.5ml of serum was pipette into 'T' test tube and 0.5ml of distilled water into test tube 'B'. Then 0.5ml of 40% TCA and 1ml of 0.67% TBA was added to each. The contents in the tube were mixed and then placed in boiling water bath for ten minutes. Then tubes were removed and cooled under ice cold water. Then mixture was centrifuged at 6000rpm for 15 minutes. Absorbance of supernatant was read at 530nm. MDA content of sample was calculated using molar extinction coefficient 1.56 X 100000 and expressed as nmol/ml of serum.

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 less than 0.01

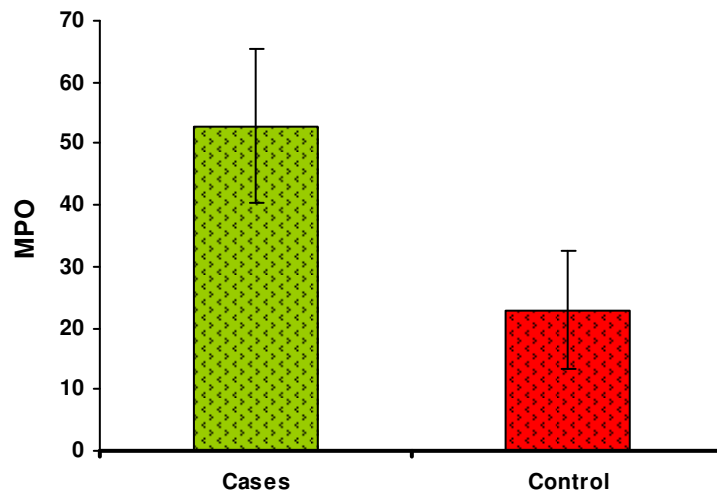
93 was considered as statistically significant. Microsoft word and Excel have been used to generate graphs
94 and tables.

95 **RESULTS:** It is a cross sectional study. Fifty PE women and fifty normal pregnant women were
96 our study subjects. Enzyme activities of MPO and serum levels of MDA were estimated to determine
97 the oxidative stress in PE and compared with normal pregnancy.

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

103 **Table No. 1: Range of study variables MPO and MDA among study groups**

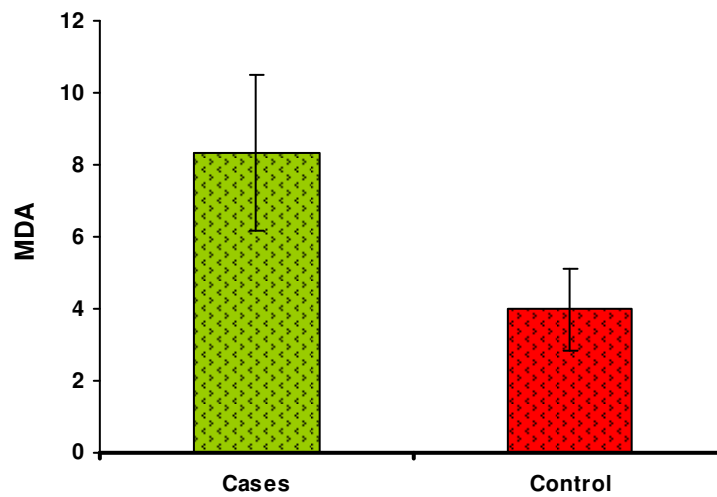
Study Variables	Cases	Control	P value
MPO (U/L)	52.79±12.57	22.91±9.53	<0.001**
MDA (nmol/ml)	8.33±2.16	3.98±1.13	<0.001**



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105 **Figure No. 1: Comparison of MPO activity among study groups**

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108 **Figure No.2: Comparison of MDA levels among study groups**

109 **DISCUSSION:** Preeclampsia is commonest disorder of pregnancy whose etiology is multifactorial.

110 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 PE. In this regard several authors have studied the oxidative stress in PE by estimating various oxidants and antioxidants. We have studied MPO a pro-oxidant and MDA a lipid peroxidation product and a marker of oxidative stress in the PE women and compared with the normal pregnant women.

PE and normal pregnant woman were age matched. Majority of the study subjects were primigravida (78% of the PE women and 64% of normal pregnant women).

In our study mean MPO level of cases was 52.79 ± 12.57 U/L and controls was 22.91 ± 9.53 U/L. Thus we observed significant ($P < 0.001$) increase in the MPO levels in PE women compared to normal pregnant women. Our study correlates with the Mellembakken JR et al (2001) and Robin E. Gandley et al (2008). They found significantly increased levels of MPO in the circulation of women with PE. Robin E Gandley et al in their study found plasma MPO levels 3-fold higher in patients with PE compared with normal control subjects. They also observed significantly increased MPO levels in placenta of women with preeclampsia in addition to increased serum MPO levels⁽¹⁴⁾.

MPO induces low-density lipoprotein oxidation, activates metalloproteinases, and oxidatively consumes endothelium-derived NO.

MPO being versatile enzyme react with several other substrates like polyphenols, catecholamine, aminoacids, superoxide, nitrite, ascorbic acid (vitamin C) and uric acid beside chloride. The oxidation of these substrates generates free radicals intermediates, organic hydroperoxides, reactive aldehydes and other oxidant products that are able to react with structural and functional molecules in the host like proteins, membrane lipids and nucleic acids and cause tissue damage. The oxidation products of MPO like oxidized LDL, oxidized apolipoprotein (apo) A1, HDL are responsible for

the progression of inflammation and tissue injury seen in the causation and progression of several diseases including PE.

MPO may also has destabilizing effects on atheromatous plaque through the activation of metalloproteinases, induction of endothelial apoptosis, and stimulation of tissue factor expression.

MPO is also capable of using nitric oxide (NO) an endothelium- derived relaxing factor as a substrate to catalyze tyrosine nitration in proteins thereby serving as a catalytic sink for nitric oxide contributing to endothelial dysfunction which is central to the etiology of PE.

Thus MPO has plausibly been proposed to contribute several phases of atherothrombosis seen in preeclampsia, from the initial insult to the vascular endothelium, to development of atheroma, to rupture of vulnerable atherosclerotic plaque and its clinical manifestations⁽¹⁵⁾.

Several investigators have found that MPO activity, increased in direct proportion to the severity of PE. This increase in the serum MPO activity is probably the result of an enhanced systemic inflammatory reaction. In our study about 30% of the PE women were found to have normal MPO activity similar to normal pregnant women. This may be due to early diagnosis and treatment which has probably modified the inflammatory process. This study has demonstrated that, MPO an oxidant plays an important role in PE. Elevated levels of plasma MPO serve as a marker of increased risk of PE.

In the present study mean MDA levels of cases was 8.33 ± 2.16 nmol/ml and controls was 3.98 ± 1.13 nmol/ml, we observed significant ($P < 0.001$) increase in MDA levels in preeclamptic women compared to normal pregnant women. Our findings are in accordance with J.B.Sharma et al (2006), AyseNurAksoy et al (2008), K.S.Meera et al (2010)⁽¹⁶⁾. In the other words, MDA is a marker of oxidative stress.

Despite substantial advances in understanding the pathogenesis of PE, the development of simple tests to identify individuals or populations at risk still remains a epidemiologic, and clinical challenge. Estimation of panel of oxidative stress markers like MPO and MDA in the early pregnancy may help in the identifying pregnant women at risk of developing PE and execution of preventive measures.

CONCLUSION: Preeclampsia is a common complication of pregnancy whose etiology is still unknown. Oxidative stress is one of the major mechanisms considered for the etiopathogenesis of PE. The results of this study show that there is an increase in oxidant status in PE leading to oxidative stress which further causes endothelial damage and signs and symptoms of PE. Early detection and timely intervention can prevent the development of PE.

Limitation of the study: Sample size; prospective study should be carried out in larger number of pregnant women. This study included both treated and untreated preeclamptic women; treatment may modify the disease process.

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