COMPARATIVE STUDY OF OXIDATIVE STRESS IN PREGNANCY INDUCED HYPERTENSION PREECLAMPSIA AND ECLAMPSIA

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Abstract

Background and Objective: A Hypertensive disorder of pregnancy is an important cause of maternal mortality and preeclampsia. The present study is centered on the concept that a derangement if any, in the oxidant antioxidant equilibrium during Pregnancy Induced Hypertension, Preeclampsia and Eclampsia.

Materials and Methods: Defined groups of Pregnancy Induced Hypertension, Pre-eclampsia and eclampsia patients were selected with written informed consent with fifty subjects in each group. Blood sample was collected by venous puncture and centrifuged to get serum. The estimation of Malondialdehyde, Superoxide dismutase and catalase in three different groups was done using standard spectrophotometric method. The data were expressed as Mean ± SD. Comparison of oxidative stress between patients and controls was performed using unpaired t test. P value less than 0.05 was considered significant.

Results: The level of MDA was significantly increased in (P<0.001) in PIH group but, the level of SOD and Catalase was significantly less (P<0.01). The level of MDA and Catalase in preeclampsia was significantly increased (P<0.001) but, the activity of SOD was decreased significantly (P<0.001). Similarly, in preeclampsia and Eclampsia group. When the serum MDA and catalase activity in erythrocytes and SOD level in PIH, Preeclampsia and Eclampsia were compared with normal group has shown a significant increase (P<0.001) in MDA and Catalase but significant decline (P<0.001) in SOD level respectively in all the groups.

Conclusion: In PIH, Preeclampsia and Eclampsia there is an imbalance between lipid peroxides and the antioxidant system.

Keywords: Pregnancy Induced Hypertension, Pre-eclampsia, Eclampsia, Malondialdehyde, Superoxide dismutase, Catalase

1. Introduction:
Hypertensive disorders of pregnancy are an important cause of maternal mortality and preeclampsia accounts for more than 40% of iatrogenic premature deliveries. Despite the high cost to families and health service resources, there is no effective management strategy other than elective deliveries and no therapeutic interventions have been proven to prevent or delay the onset of this disease. There is increasing evidence that oxidative stress is an important contributing factor to the pathogenesis of preeclampsia. Considerable attention has been focused on the relationship between hyper oxidant stress and toxemia of pregnancy in the field of obstetrics and gynecology. Free radicals derived from molecular oxygen and nitrogen is highly reactive metabolites called reactive oxygen species. Cells continuously produce free radicals and reactive oxygen species as part of the metabolic process. Free oxygen radicals in the body cause various types of damages like Peroxidation and subsequent endothelial cell injury and damage to proteins and nucleic acids in mother’s placenta, brain liver kidney etc.
Antioxidants are enzymes or compounds that scavenge and reduce the presence of free radicals. Cells and tissues are usually protected from the effects of lipid Peroxidation by general naturally occurring antioxidants. They block free radical damage to cell membranes, Nerves and cholesterol. Normally, a balance exists between concentrations of reactive oxygen species and antioxidant scavenging system. The disruption of the delicate balance between pro and antioxidants results in oxidative stress.

During pregnancy there is an increase in cellular metabolism and therefore, an increase in oxidative stress and lipid Peroxidation compared with nonpregnant women and even worse in preeclamptic women. Maker of lipid Peroxidation like malondialdehyde is increased in the plasma of women with preeclampsia, and the low concentration of water soluble and lipid
soluble antioxidants in the plasma further suggest state of oxidative stress.
The term Pregnancy Induced Hypertension (PIH) is used to describe any new onset pregnancy related hypertension. This designation served to emphasize the cause and effect connection between pregnancy and a unique form of hypertension which manifest in women only during reproduction. PIH would include the development of hypertension without proteinuria including in nulliparous women. The term PIH is also transient uncomplicated hypertension that subsided promptly after delivery. PIH was also a potential precursor to preeclampsia or eclampsia, which require proteinuria for diagnosis. The development of hypertension in a previously normotensive pregnant woman should and must be considered potentially dangerous to both the mother and her fetus.
Preeclampsia is a pregnancy specific syndrome of reduced organ perfusion secondary to vasospasm and endothelial activation. It is associated with hepatic, neurological, hematological and renal involvement. Rapid development of edema, particularly of the face and hands, along with a rise in blood pressure, often signals the onset of this condition. Jaundice and abnormal liver functions may be present.
Eclampsia is the occurrence of seizures in a woman with preeclampsia that cannot be attributed to other causes. The seizures are grand mal and may appear before, during or after labor. Seizures that develop more than 48 hrs postpartum however, especially in nulliparous may be encountered up to 10 days postpartum. The present study is centered on the concept that a derangement if any, in the oxidant antioxidant equilibrium during Pregnancy Induced Hypertension, Preeclampsia and Eclampsia.

2. Materials and Methods:
The patients for the present study were from the Out Patient ward and Eclampsia room in the institute of Maternal and Child Health, Medical College, Calicut. The study was approved by institutional ethics committee. Defined groups of pregnancy induced hypertension; Pre-eclampsia and eclampsia were selected with prior written informed consent. The total study group is divided into Normal Pregnancy (3rd trimester), PIH, Pre-eclampsia and Eclampsia with fifty subjects in each group. Blood samples were collected by venous puncture using disposable syringes and needles and transferred into clear dry centrifugation at 3000rpm for 15 minutes. For estimation of catalase, blood was collected in bottles containing heparin and plasma was separated. Chemicals used for the estimation were of analytical quality obtained from sigma, BDH and Merck. Analysis was done in UV-Vis spectrophotometer 118 (Systronics). Parameters selected to assess the oxidant stress in PIH, preeclampsia and eclampsia were Serum MDA level and antioxidant enzymes like Superoxide dismutase in serum and Catalase in erythrocytes.

2.1 Estimation of malondialdehyde: Malondialdehyde (MDA) was measured in serum by the method based on Vignesh K and Sadasivadu’s procedure for estimation of Malondialdehyde. In brief, added 1 ml of serum to 1ml 40% trichloroacetic acid followed by addition of 2ml of 0.67% of thiobarbituric acid. The mixture was kept for 10 minutes in boiling water bath. It was cooled immediately in ice cold water bath. The mixture was centrifuged at 6000rpm for 15 minutes. Absorbance of supernatant was read against distilled water blank at 530nm.

2.2 Estimation of catalase: Superoxide dismutase (SOD) activity was measured by the method suggested and modified by Nandi et al 1988. In brief, to 2.8ml of tris buffer, 0.1ml of sample was added, mixed and started the reaction by adding 0.1ml adjusted pyrogallol solution (as per control). O.D was read at 420nm exactly after 1 minute 30 second and 3 minute 30 second and recorded the absorbance per 2 minutes.

2.3 Estimation of catalase: Blood was collected in the heparinized container. Removed the supernatant plasma and centrifuged. WBCs removed from the top layer. RBC washed with saline twice. Blood was made to hemolyse by adding 1.5 volumes of ether. Measured the hemoglobin concentration and adjusted to 5gm%. Hemoglobin was estimated by using cyanmethaemoglobin method of Drabkin. A 1,500 dilution of this concentrated haemolysate was prepared with phosphate buffer immediately before the assay. Two tubes, one blank and one test were taken and 2ml of phosphate buffer and to the test, 1 ml of H2O2 was added. The decrease in extinction was followed at 5 second intervals for 15 seconds. The rate constant K of the 1st order reaction was calculated and K.ml of blood was used as a measure of specific activity of catalase.
2.4 Statistical analysis: The data were expressed as Mean ± Standard Deviation. Baseline parameters were compared using Student's t-test. Comparison of oxidative stress between patients and controls was performed using unpaired t-test. P value less than 0.05 was considered significant.

3. Results:
The present study evaluates the role of oxidative stress in PIH preeclampsia and eclampsia. This is done by measuring the levels of Malondialdehyde and superoxide dismutase in serum and catalase activity in erythrocytes. Age, gestational age, systolic and diastolic blood pressures and protein creatinine ratio in urine has also been included as baseline parameters. The results are tabulated in Tables 1-6.
The mean value of MDA was found to be significantly increased (P<0.001) in PIH group. Mean SOD and Catalase in PIH group was found to be significantly less (P<0.01) than that of the normal group (Table-1). The mean values of MDA and Catalase in preeclampsia was found to be significantly increased (P<0.001) as compared to normal pregnant. Whereas, the activity of SOD was decreased significantly (P<0.001) in patients suffering from preeclampsia (Table-2). The result of MDA, SOD and Catalase was similar to preeclampsia in Eclampsia (Table-3). When the serum MDA and catalase activity in erythrocytes in PIH, Preeclampsia and Eclampsia were compared with normal group has shown a significant increase (P<0.001) respectively in all the groups (Table-4and 5). Whereas, the activity of SOD in PIH, Preeclampsia and Eclampsia when compared with normal group has shown a significant decline (P<0.001) respectively in all the groups (Table-6).

4. Discussion:
The present study was conducted to assess the role of oxidative stress in hypertensive disorders of pregnancy by comparing the levels of lipid peroxide product Malondialdehyde and the activities of antioxidant enzymes – SOD and catalase between normal pregnant women and those with PIH, preeclampsia and eclampsia. Serum MDA levels were significantly increased in PIH, preeclampsia and eclampsia compared to normal third trimester pregnant women. Jain SK and Wise RY Onekama et al obtained similar results. Wang YP et al, Gratacos E et al, Madazli R et al found increased levels of lipid peroxides in PIH and preeclampsia compared to controls. The findings of Kumar CA and Das UN and Pyska W et al were consistent with previous studies suggesting that lipid peroxidation is an important factor in the pathogenesis of preeclampsia. Increased activation of neutrophils, macrophages and T cells along with exaggerated placental response resulting in elevated formation of reactive oxygen species like superoxide radical, hydroxy radical which will cause increase in lipid peroxidation damage to vascular endothelium, membranes of cells and organelles which is evidenced by elevation of MDA level.
The levels of serum SOD were found to be significantly decreased in patients with PIH, preeclampsia and eclampsia compared to controls. Davodge ST et al found that antioxidant activity was markedly reduced in preeclampsia. The excessive generation of free radicals inactivates the enzymes system in the body leading to decreased SOD activity observed in present study. In this study, a significant increase was observed in catalase activity in PIH, preeclampsia and eclampsia and eclampsia compared to controls. Wang Y and Walsh SW described high catalase activity in placental tissues in women with preeclampsia, the most likely explanation being stimulation of antioxidant capacity in response to a marked increase in oxidative stress. Since the above mentioned studies and the present study are not showing any relation in the catalase activity in preeclampsia, further studies are needed to know whether these hypertensive disorders are having any influence upon the synthetic and degradative pathway of catalase enzyme protein.

5. Conclusion:
The present study has shown a significant increase in serum MDA levels, the indicator of lipid peroxidation, in the 3 groups suggest that lipid peroxidation plays a role in the pathogenesis of hypertensive disorders of pregnancy. Increased lipid peroxidation causes increased consumption of antioxidant free radical scavenging system. In PIH, preeclampsia and eclampsia there is an imbalance between lipid peroxides and the antioxidant system.
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### Table 1: Comparison of serum MDA, SOD and Catalase in control group and pregnancy induced hypertension.

|                          | Normal          | Pregnancy Induced Hypertension | P-Value |
|--------------------------|-----------------|-------------------------------|---------|
| MDA (nmol/dl)            | 81.97 ± 26.43   | 109.09 ± 24.16                | <0.001  |
| SOD (U/ml)               | 4.17 ± 1.65     | 3.24 ± 1.32                   | <0.01   |
| Catalase (K/ml)          | 4.24 ± 2.10     | 6.95 ± 2.45                   | <0.01   |

Values are expressed as Mean ± SD. N=50 in each group.

### Table 2: Comparison of serum MDA, SOD and Catalase in control group and Preeclampsia.

|                          | Normal          | Preeclampsia                  | P-Value |
|--------------------------|-----------------|-------------------------------|---------|
| MDA (nmol/dl)            | 81.97 ± 26.43   | 130.65 ± 43.02                | <0.001  |
| SOD (U/ml)               | 4.17 ± 1.65     | 2.69 ± 1.34                   | <0.001  |
| Catalase (K/ml)          | 4.24 ± 2.10     | 7.10 ± 2.76                   | <0.001  |

Values are expressed as Mean ± SD. N=50 in each group.

### Table 3: Comparison of serum MDA, SOD and Catalase in control group and Eclampsia.

|                          | Normal          | Eclampsia                     | P-Value |
|--------------------------|-----------------|-------------------------------|---------|
| MDA (nmol/dl)            | 81.97 ± 26.43   | 141.04 ± 50.95                | <0.001  |
| SOD (U/ml)               | 4.17 ± 1.65     | 2.59 ± 1.33                   | <0.001  |
| Catalase (K/ml)          | 4.24 ± 2.10     | 7.22 ± 2.99                   | <0.001  |

Values are expressed as Mean ± SD. N=50 in each group.

### Table 4: Comparison of serum MDA levels in Pregnancy Induced Hypertension, Preeclampsia and Eclampsia with normal group.

|                          | Normal         | PIH                          | PREECLAMPSIA | Eclampsia | P Value |
|--------------------------|----------------|------------------------------|--------------|-----------|---------|
| MDA (mol/dl)             | 81.97±26.43    | 109.09±24.16                 | 130.65±43.02 | 141.04±50.95 | <0.001  |
| P Value                  |                |                              |              |           |         |

Values are expressed as Mean ± SD. N=50 in each group.

### Table 5: Comparison of catalase activity in erythrocytes in Pregnancy Induced Hypertension, preeclampsia and Eclampsia with normal group.

|                          | Normal        | PIH                          | PREECLAMPSIA | Eclampsia | P Value |
|--------------------------|---------------|------------------------------|--------------|-----------|---------|
| Catalase (K/ml)          | 4.24 ± 2.10   | 6.95 ± 2.45                  | 7.10 ± 2.76  | 7.22 ± 2.99 | <0.001  |
| P Value                  |               |                              |              |           |         |

Values are expressed as Mean ± SD. N=50 in each group.

### Table 6: Comparison of serum SOD levels in Pregnancy Induced Hypertension, Preeclampsia and Eclampsia with normal group.

|                          | Normal       | PIH                          | PREECLAMPSIA | Eclampsia | P Value |
|--------------------------|--------------|------------------------------|--------------|-----------|---------|
| SOD (U/ml)               | 4.17 ± 1.65  | 3.24 ± 1.32                  | 2.69 ± 1.34  | 2.59 ± 1.33 | <0.001  |
| P Value                  |              |                              |              |           |         |

Values are expressed as Mean ± SD. N=50 in each group.