CORRELATION BETWEEN LIPID PEROXIDATION PRODUCT- MALONDIALDEHYDE (MDA) AND REDUCED GLUTATHIONE (GSH) IN PREECLAMPSIA

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ABSTRACT: Pregnancy is a stressful condition in which many physiological and metabolic functions are altered to considerable extent and hypertension is the most commonest problem encountered during pregnancy, complicating 5-10% of pregnancies. Recent reports suggest that free radical induced endothelial damage as an important factor in the pathogenesis of preeclampsia. Such cell injury might in turn is counteracted by the action of several in vivo antioxidants. But because of increased lipid peroxidation and increased demand of antioxidants, increased oxidative stress is suspected. The present study was undertaken to determine serum malondialdehyde (MDA) and RBC reduced glutathione (GSH) levels in clinically diagnosed preeclamptic women (n=30) and compared with that of normotensive pregnant women and to find out any association between the two parameters. Serum MDA levels were significantly elevated (p value< 0.000) and RBC reduced GSH levels were significantly decreased ( p value <0.000) compared to that of normotensive pregnant women. A statistically significant negative correlation was observed between serum MDA and RBC reduced GSH (p value <0.003) in preeclamptic cases.

Key words: MDA, Reduced GSH, Preeclampsia

INTRODUCTION
Preeclampsia is a pregnancy complication recognised by new onset gestational hypertension and proteinuria. The disease affects mothers and their infants. Once the disease is evident clinically, it can be cured only by delivery (Tang LC et al, 1997). Lipid peroxidation is an oxidative process that normally occurs at low levels in all cells and tissues. Under normal conditions a variety of antioxidant mechanisms serve to control this peroxidative process (Sies H, 1986). However, in disease states such as preeclampsia an imbalance between peroxidation and antioxidant mechanisms could impair the normal endothelial function. Serum lipid peroxidation products are increased in pregnant women and this increase is further augmented in preeclampsia patients with decreased antioxidant levels (Ishihara M et al, 1978). Hence, the present study was undertaken to determine serum MDA (malondialdehyde) levels, as a marker of oxidative stress and reduced Glutathione (GSH) as a marker of antioxidant and to find out any association between these two parameters in preeclampsia.

MATERIALS AND METHODS:
Thirty cases of Preeclampsia and thirty Normotensive Pregnant women attending antenatal clinic at Narayana General Hospital, Nellore were enrolled for the study after taking informed consent. Both cases and controls were primigravida, between 18 – 30 yrs of age and were in their third trimester.
Inclusion criteria: Women with Preeclampsia diagnosed based on definition of American College of Obstetricians and Gynecologists (ACOG)s : 1) Systolic Blood Pressure greater than 140 mm of Hg or rise of at least 30 mm of Hg or 2) Diastolic Blood Pressure greater than 90 mm of Hg or rise of at least 15 mm of Hg (manifested on two occasions at least 6 hrs apart) and 3) Proteinuria of 300 mg or greater in 24 hrs urine collection or protein concentration of 1 gm/litre (on two occasions at least 6 hrs apart) (Patil Sadanand B et al 2012). Subjects with normal pregnancy were normotensive and had no proteinuria.
Exclusion criteria: Illness like anemia, diabetes mellitus, essential hypertension, renal insufficiency, cardiovascular disease, tobacco addiction which by themselves are known to alter free radical status were excluded from study. Fasting Venous Blood samples were collected into EDTA tube and plain tube.

Isolation of erythrocyte and haemolysate preparation
The blood samples were centrifuged at 1000 g for 15 minutes at 4ºC and the isolated red cells were washed 4-5 times with 0.154 M NaCl to remove plasma and buffy coat. After the final wash, the required packed cells were lysed by hypotonic shock and different dilutions were used as haemolysate.

Estimation of reduced GSH
Packed red cells (0.2ml) were used in the assay. GSH was made to react with 5, 5 –dithiobis (2-nitrobenzoic acid) , which reacts with sulphhydryl groups, to develop a stable colour. The absorbance was measured at 412 nm and GSH content expressed as micromoles / g of Hb (Mohd.Suhail et al., 2008).

Hemoglobin estimation
Haemoglobin content of the erythrocyte was measured using cyamethemoglobin method.

Estimation of MDA
Serum was used for determination of Malondialdehyde (MDA), a lipid peroxidation product, by Thiobarbituric acid reactive substances (TBARS) method (Sharma J.B et al, 2006).

Statistical analysis: Data was analysed using statistical software SPSS version 20. Values are expressed as mean ± SEM (standard error of mean). Comparison of values between cases and controls was done using Student’s t test. Correlation between two parameters was done using Pearson’s correlation test. A p value of less than 0.05 was considered statistically significant.

Table 1: Comparison of Serum MDA and RBC reduced GSH levels in Preeclampsia and Normotensive pregnant women

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Preeclamptic women (n=30) Mean ± SEM</th>
<th>Normotensive pregnant women (n=30) Mean ± SEM</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum MDA (nmol/ml)</td>
<td>24.4 ± 2.3</td>
<td>7.9 ± 0.28</td>
<td>0.000*</td>
</tr>
<tr>
<td>RBC reduced GSH</td>
<td>3.8 ± 0.174</td>
<td>6.4 ± 0.256</td>
<td>0.000*</td>
</tr>
<tr>
<td>(micromoles/g of Hb)</td>
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</tbody>
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*P value < 0.05 statistically significant, n= number of subjects

Figure 1: Scattered diagram showing correlation between Serum MDA and RBC reduced GSH in Preeclampsics
Note: RBC reduced GSH expressed in micromoles/g of Hb and Serum MDA expressed in nanomoles/ml.

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RESULTS AND DISCUSSION
Preeclampsia can have significant impact on health of both mother and fetus. In present study, we observed that mean serum MDA levels were significantly increased in preeclamptic women as compared to that of normotensive pregnant women (p value < 0.000). Similar observations were noted by others (Kashinakunti SV et al, 2010, Sheena PS et al, 2010, Shikha Saxena et al, 2014). The lipid peroxides and free radicals may be important in the pathogenesis of preeclampsia (Mohan KS et al, 2007, Hubel CA et al, 1989).
Glutathione and glutathione related enzymes are one of the major antioxidant systems within body. GSH is the most abundant thiol-based antioxidant, and is found primarily in the reduced form, with intracellular concentrations up to 11 mM and provides sulfhydryl−buffering capacity. It also conveys antioxidant power through the direct inactivation of ROS or by acting as an electron donor for glutathione peroxidase that reduces H2O2 to water (Imai H, 2003). It has been suggested that the high levels of GSH may act as compensatory mechanism to prevent excessive lipid peroxidation via membrane bound glutathione peroxidase (Knapen MF et al, 1999). In our study, the mean RBC reduced GSH levels were significantly decreased in preeclampsia cases when compared to that of normotensive pregnant women (p value <0.000). Similar observations were noted by others (Mossa M. Marbut et al, 2009, Mohd. Suhail et al, 2008, Yildiz Atamer et al, 2005). We also noted that there was statistically significant negative correlation between MDA levels and reduced GSH levels in preeclamptic patients (r value -0.528, p value <0.003), similar to other studies (Padmini E et al, 2008, Yoshio et al, 2002). The decrease in this nonenzymatic parameter with parallel increase in MDA, may be due to the increased turnover, for preventing oxidative damage in these patients suggesting an increased defence against oxidant t damage in preeclampsia (Ozan H et al 2002, Mohan KS et al 2007).

CONCLUSION
We conclude that preeclampsia is associated with increased free radical generation and a fall in antioxidants like reduced GSH, reflecting enhanced oxidative stress in these patients. Antioxidant therapy with vitamin C may be helpful to combat oxidative burden and minimize the free radical damage in preeclamptic patients.

REFERENCES


