



## **Oxidative stress and antioxidants levels in normotensive and pre-eclamptic pregnant women in their third trimester pregnancy**

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### **Abstract**

**Background** - Oxidative stress is defined as a imbalance between the cellular generation of reactive oxygen species (ROS) and the capacity of antioxidants to prevent oxidative damage. Preeclampsia is a complex systemic disorder that includes elevated arterial blood pressure, edema and proteinuria. The enhanced activity of Super oxide dismutase during pregnancy acts to counter the placental generation of ROS, promoting a higher anti oxidant status in normal pregnancy and preventing oxidative stress.

**Materials and Methods** – A total of 200 pregnant women (in their III<sup>rd</sup> trimester) age group between 20-35 years were selected. All the subjects were categorised under 2 groups viz, control group (Normotensive pregnant women) and study group (pre eclamptic pregnant women). Their serum SOD, Vitamin C & E levels were estimated.

**Results** – Levels of SOD, vitamin C & E were lower by 23%, 40% and 28% respectively in our study group as compared to control groups.

**Conclusion** – Decrease levels of antioxidants (SOD, Vitamin C & E) may be the cause of pregnancy induced hypertension or preeclampsia.

**Keywords** – Oxidative Stress, Preeclampsia, SOD, Vitamin C & E

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## Introduction

Oxidative stress is defined as an imbalance between the cellular generation of reactive oxygen species (ROS) and the capacity of antioxidants to prevent oxidative damage<sup>1</sup>. Antioxidant defences appeared to be depleted during preeclampsia resulting in oxidative stress<sup>2-5</sup>.

Preeclampsia is a complex systemic disorder that includes elevated arterial blood pressure, edema and proteinuria<sup>6</sup>. ROS can cause cellular damage by oxidizing nucleic acids, proteins and membrane lipids<sup>7</sup>. They may also influence vascular toxicity either indirectly by inactivating the endothelium derived relaxing factor, which is nitric oxide and reducing the release of prostacyclin or directly by containing smooth muscles<sup>8</sup>. Thus uncontrolled lipid peroxidation may play an important role in the pathophysiology of pre eclampsia. Placental oxidative stress is controlled by the interception destruction or modification of ROS through the action of antioxidants system. These antioxidant systems can be divided into two major groups – enzymatic (superoxide dismutase) and non enzymatic (Vitamin C & E). These low molecular weights compounds scavenge superoxide, hydrogen peroxide and hydroxyl radicals preventing excessive biological oxidation and cellular damage. Vitamin C & E are efficient antioxidants and their use in pregnancy, and in particular prevention of preeclampsia<sup>9,10</sup>. Superoxide dismutases are in the first line of antioxidant defence and are responsible for metabolizing two molecules of superoxide (O<sub>2</sub><sup>-</sup>) to produce H<sub>2</sub>O<sub>2</sub> and molecular oxygen. SODs contains redox active metal ions e.g. Copper, Zinc, Manganese in their catalytic centre and exists in three different isoforms. All three forms of SOD are expressed in placental tissues. The enhanced activity of SOD during pregnancy acts to counter the placental generation of ROS, promoting a higher anti oxidant status in normal pregnancy and preventing oxidative stress. The deficient SOD activity in the placental compartment during pregnancy would lead to increased production of superoxide and the generation of oxidized lipids, proteins and DNA. SOD modulates trophoblast differentiation and the decrease expression during preeclampsia would lead to enhanced syncytialisation leading to increased apoptosis and trophoblast turnover. All these changes have been observed in the pre eclamptic placenta<sup>12-15</sup>.

## Material and Methods

This study was performed on 200 pregnant women (in their III<sup>rd</sup> trimester) age group between 20-35 years. All the subjects were categorised under 2 groups viz, control group (Normotensive pregnant women) and study group (pre eclamptic pregnant women).

For the biochemical parameters to be analysed, blood samples were collected after an overnight fast from the antecubital vein avoiding venostasis in all subjects. EDTA vials were used for the estimation of antioxidants vitamin (E & C) and Enzyme (SOD).

Blood samples were immediately centrifuged at 3000 rpm for 10 min, plasma was collected and analysis was done immediately after collection. Plasma vitamin C levels was determined spectrophotometrically by Carl A Burti's method, plasma vitamin E levels was determined by the method of Emmeric-Engel.

**Blood lysate proportion** whole blood (1 ml) was aspirated and erythrocytes were washed four times with 0.9% NaCl solution (3.0 ml). After each wash the erythrocytes were centrifuged for 10 min at 3000rpm and the supernatant was discarded. The washed erythrocytes were diluted upto 2.0ml with cold distilled water and left to stand at 4°C for 15 minutes. The lysate was further diluted 25 times and the diluted lysate was used for the determination of antioxidant enzyme. This procedure removed more than 87% of leucocytes.

**Enzymatic determination of Antioxidant Enzyme** SOD (EC:1:15:1:1) activity in erythrocytes was measured by the rate of auto oxidation of pyrogallol by SOD, as described by Marklund and Marklund method. Enzyme activity was expressed as unit/gm haemoglobin (u/g Hb). Haemoglobin percentage was estimated by Beutler's method. Double

beam UV/Vis spectrophotometer was used for the determination of the each sample. Statistical Analysis

Values are expressed as Mean± SD. The significant mean difference between groups were assessed by the “student t” test and distribution of t’ probability (p).

## Result

The observation revealed the changes in antioxidants (Vitamins and Enzyme) levels in Normotensives and preeclamptic pregnant women in their third trimester pregnancy are represented as follows

- Enzyme Superoxide dismutase levels in study group showed highly significant ( $p < 0.001$ , 23.1%) as compared to control group (Normotensive group) (Table 1)
- Vitamin C levels in study group (pre eclamptic group) showed significant decrease ( $p < 0.001$ , 40.3%) (Table 2)
- The percentage decrease in vitamin E levels as compared to controls was 28.3%. Statistically the changes observed were highly significant ( $p < 0.001$ ) in study group as compared to controls. (Table 3)

Table 1- Superoxide dismutase (SOD) levels in Normotensives and pre eclamptic group. Expressed as (u/gHb)

Sl No.	Particulars	Normotensives	Pre-eclamptic
1	Mean± SD	1670.84 ± 230.30	1284.71± 154.99
2	% Decrease		↓23.1%
3	t value		9.61
4	P value		<0.001

Table 2- Vitamin C levels in Normotensives and preeclamptic group Expressed as (mg/dl)

Sl No.	Particulars	Normotensives	Pre-eclamptic
1	Mean± SD	0.82± 0.41	0.49± 0.23
2	% Decrease		↓40.3%
3	t value		4.75
4	P value		<0.001

Table 3- Vitamin E levels in Normotensives and preeclamptic group Expressed as (mg/dl)

Sl No.	Particulars	Normotensives	Pre-eclamptic
1	Mean± SD	0.92± 0.25	0.66± 0.26
2	% Decrease		↓28.3%
3	t value		5.01
4	P value		<0.001

## Discussion

Reduction in SOD levels in our study could be attributed to more consumption of SOD in combating free radicals mediated oxidative stress in the hypertensive proteinuric syndrome. This theory of oxidative stress in the form of lipid peroxidation was proposed for the first time by Hubel et al 8 in 1989, Witztum<sup>16</sup> in 1994 as well by others<sup>17-20</sup>. Preeclampsia was also shown to be associated with it. Several studies have demonstrated decreased plasma levels of Vitamin C and E compared to normal pregnant women. Similarly, in our study we observed a significant decreased in plasma levels of Vitamin E and C in the pre eclamptic

pregnant women. The percentage decreased in vitamin E and C from normotensive subjects was 28.3% and 40.3% respectively. Fetoplacental unit may be origin of oxygen free radicals and lipid peroxides<sup>21, 22</sup>. Vascular contact with placental originated circulating peroxidation products may cause dysfunction of the vascular endothelium by promoting peroxidative damage of endothelial cell membranes. Such events established a cycle ultimately leading to manifestation of pre eclampsia.

Since antioxidants deficiency is a cause of lipid peroxide accumulation, vitamin E and C therapy may alter the disease process. It initiated in early gestation to patients at risk.

Kumar and Das<sup>23</sup> showed that the activities of erythrocytes SOD and Vitamin E were significantly decreased in patients with preeclampsia compared to normotensive pregnant women. Low values of serum SOD in pre eclampsia were first shown by Bayhan et al<sup>24</sup> in 2000, Kharb et al<sup>25</sup> in 2000, Davidge<sup>26</sup> in 1998 and Hubel et al<sup>27</sup> in 1999.

### **Conclusion**

Our findings demonstrate that some antioxidant systems, such as the SOD and the vitamins (C & E ) have been demonstrated to be reduced in red blood cells in pre eclampsia. Hence the present investigation still suggest that the supplements of the antioxidants or free radical scavengers provides against oxidative stress, acts by restoring the enzymes activity and preventing the further damage cause by lipid peroxidation.

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