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Levels of Upper Limit of Oxidative Stress Markers During Normal Pregnancy And Pre-Eclampsia

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ABSTRACT

Background: *The reactive oxygen species (ROS) are involved in multiple reproductive processes – maturation of oocyte, fertilization, development of embryo, pregnancy and parturition. However, the optimal levels and interplay of oxidants and antioxidants is crucial for a positive pregnancy outcome. Pregnancy induced hypertension (PIH) is shown to have defective trophoblast invasion leading to high pressures in utero-placental circulation; thus this stress imposed on placenta may disturb the oxidant-antioxidant balance.*

Aim: *the purpose of this study was to assess the oxidative stress during uncomplicated pregnancy and pregnancy associated with hypertension in primi-gravida women.*

Materials And Methods: *a total of 90 women were enrolled and divided into 3 groups. Group 1: Healthy non-pregnant women (n=30), Group 2: Normotensive primi-gravida women (n=30) and Group 3: Pre-eclamptic primi-gravida women (n=30). Blood samples were analyzed for levels of malondialdehyde (MDA), superoxide dismutase (SOD), total anti-oxidant capacity (TAC) and uric acid using appropriate biochemical methods. ANOVA was used for assessing the difference between the groups and statistical*

significance was fixed at p<0.05.

Results: *In the present study, levels of MDA, SOD and uric acid were significantly increased in pregnant compared to non-pregnant women and to a greater extent in pre-eclampsia women compared to normal pregnant women.TAC levels were significantly decreased in pregnant when compared to non-pregnant and much more decreased in pre-eclampsia women compared to normal pregnant women.*

Conclusion: high pressures in feto-placental circulation can lead to increased elaboration of oxidative radicals which may cause oxidative damage to placenta and other organs.

Keywords: *oxidative stress, pregnancy, pre-eclampsia*

INTRODUCTION

Reactive oxygen species (ROS) and Reactive nitrogen species (RNS) are the two major types of free radical species characterized by high reactivity and instability[1].Studies have established the role of free radicals (in low/moderate concentrations) in various physiological functions like vascular tone regulation, signal transduction, maintenance of redox homeostasis etc. In healthy individuals, presence of a wide range of antioxidants systems either limits the production of ROSor inactivates them thereby repairing cell damage and maintaining homeostasis. Excess production of oxidants results in oxidative stress-induced cellular damage, degeneration and mutagenesis [2].

The effect of oxidative stress is seen throughout the reproductive phase of a woman and also during menopause. The reactive oxygen species (ROS) influence multiple processes ranging from maturation of oocyte to fertilization, development of embryo and pregnancy and parturition [3]. A characteristic feature observed during pregnancy is that there is an increased requirement for tissue oxidative processes which are already involved in the chain of events preceding the onset of pregnancy. The mitochondrial activity in the placenta is increased due to the production of ROS. Physiological levels of ROS like nitric oxide, carbon monoxide, superoxide anion, etc. are involved in the proliferation and differentiation of the trophoblastic tissue and vascular reactivity. Oxidative processes also have an impact on maternal cardiovascular system, resulting in an increase in the blood volume and cardiac output, and a decrease in the systemic blood pressure. Studies have shown that oxidative stress varies during pregnancy. ROS are required at different levels during the three trimesters of pregnancy for the various developmental processes. However, increased oxidative stress during first trimester can result in pregnancy loss and during second and third trimesters can result in pregnancy-related complications like intrauterine growth restriction, pre-eclampsia, gestational diabetes, etc. One possible reason for such complications is inadequate anti-oxidant response to increased oxidant levels [4].

Nitric oxide promotes relaxation of uterus during pregnancy. Experimental studies in rats have also

revealed that there is increased biosynthesis of NO which may contribute to the uterine vasodilation in pregnant rats [5].

Normal pregnancy is associated with increase in metabolism and therefore increased tissue requirement of oxygen. This results in oxidative stress with resultant increase in antioxidant response. Studies have shown a significant increase in thiobarbituric acid reactive substances (which is a by-product of lipid peroxidation) and a decrease in antioxidant activity in pregnant women when compared to non-pregnant women [6,7,8,9]. It is also associated with transformation of spiral arteries into low-resistance vessels which is responsible for increasing the blood flow for oxygen and nutrients to the growing conceptus. Defective trophoblast invasion can result in high pressure in utero-placental circulation resulting in pre-eclampsia. This condition is associated with high levels of circulating reactive oxygen species which are responsible for generation of hypertension during pregnancy [10].

Production of lipid peroxides from the placenta is increased in pre-eclampsia resulting in increased levels of malondialdehyde; and decrease levels of glutathione and superoxide dismutase in the plasma [11,12,13].

This study therefore makes an attempt to measure the oxidants and antioxidant levels in normal pregnancy and in PIH women.

MATERIAL AND METHODS

The study was carried out at the Government Maternity Hospital (GMH), Petlaburj, Hyderabad from January 2010 to July 2011. The study population comprising of 90 female subjects between the age of 20-30 years was divided into three groups - Group 1: Healthy non-pregnant women(n=30), Group 2: Normotensive primi-gravida women (n=30) and Group 3: Pre-eclamptic primi-gravida women(n=30). Women diagnosed with gestational diabetes or any other systemic disorder compromising pregnancy or general health; and those who were obese, alcoholic and anemic were excluded from this study.

Sample Collection

Under aseptic precautions, 6 ml of blood was drawn from antecubital vein by veni-puncture and collected in a heparinized tube. The sample was centrifuged at 3000 rpm for 10 minutes and the serum was collected for further biochemical analyses.

Blood Biochemistry

The levels of Malondialdehyde (MDA), Superoxide dismutase (SOD), Total antioxidant capacity (TAC) and Uric acid were analyzed using appropriate biochemical methods. Serum malondialdehyde was estimated using Thiobarbituric acid reactive substances assay (TBARS) as described by Kuno Yagi [14], serum total proteins using Biuret method [15], superoxide dismutase by inhibition of auto-oxidation of adrenaline as given by Mc Cord and Fridivich [16], TAC by FRAP (Ferric Reducing Antioxidant power) assay [17] and uric acid was measured using Uricase/PAP method [18].

Statistical Analyses

Analyses were performed using SPSS version 18.0 (PASW Statistics) for Windows (SPSS, Inc., Chicago, IL) and Origin pro 8.0. ANOVA was used to know the differences in test parameters between the 3 groups. Statistical significance was set at $p< 0.05$.

RESULTS

Table 1 gives the demographic characteristics of the study population. The levels of circulating MDA, SOD, TAC and Uric acid in the 3 groups are illustrated in table 2. In the present study, levels of MDA, SOD and Uric acid were significantly increased (Fig 1, 2, 3) in pregnant compared to non-pregnant women and significantly greater in preeclampsia women compared to normal pregnant women. However, TAC levels were significantly decreased (Fig 4) in pregnant when compared to non-pregnant and significantly decreased in preeclampsia women compared to normal pregnant women.

Table 1: Demographic characteristics of study population of non-pregnant, pregnant and pre-eclampsia women

Parameter	Non-Pregnant women	Pregnant women	Pre-eclampsia women
Age (yrs)	24±1	22±2	23±2
Weight (kg)	57±5	54±3	56±2

Values are given as mean±standard deviation

Table 2: Levels of circulating Malondialdehyde (MDA), Superoxide dismutase (SOD), Total antioxidant capacity (TAC) and Serum uric acid (UA) levels in non-pregnant, normal pregnant and pre-eclampsia women.

Parameter	Non-pregnant women	Normal pregnant women	Pre-eclampsia women
MDA (nmol %)	371.93±78.66	461.53±57.30	532.43±55.84
SOD (per gm protein)	79.55±10.69	102.89±10.02	113.32±16.11
TAC (µmol/lit)	1128.60±282.27	865.03±127.33	517.40±64.02
UA (mg/dl)	2.32±0.46	3.32±0.75	6.32±1.38

Values are given as mean ± standard deviation.

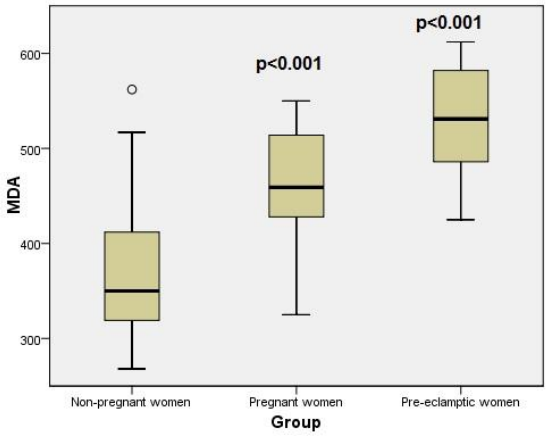


Fig 1: MDA levels

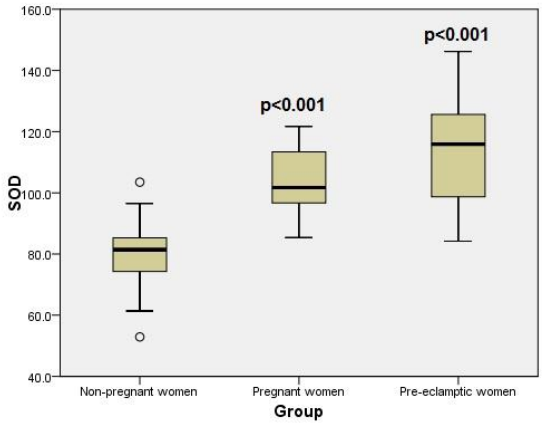


Fig 2: SOD levels

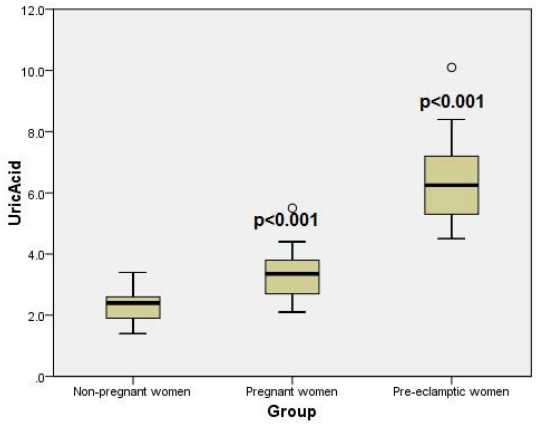


Fig 3: Uric acid levels

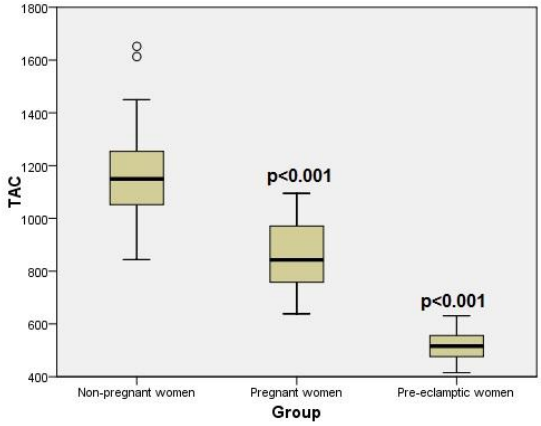
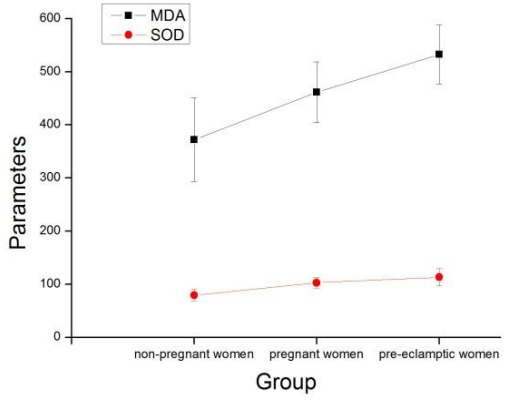
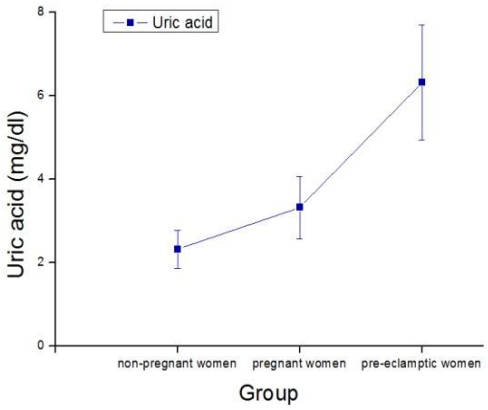
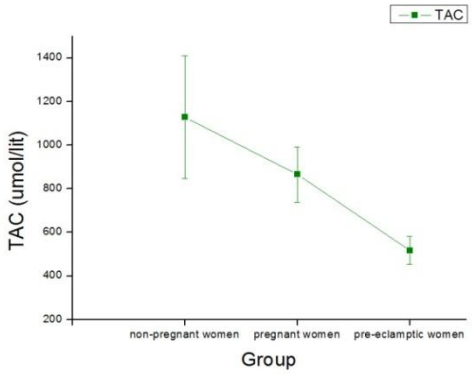


Fig 4: TAC levels







DISCUSSION

Malondialdehyde (MDA) is a product of lipid peroxidation. Oxidants affect mainly polyunsaturated fatty acids because they contain multiple double bonds. Oxidants disintegrate the double bonds and form the peroxidation product malondialdehyde. Therefore, malondialdehyde level is an indirect indicator of oxidative stress. Increased MDA levels in pregnancy thereby indicate increased production of oxidants during pregnancy. In previous studies, significant increase in levels of MDA was observed in pregnant women as compared to non-pregnant controls [6,8,9]. Serum concentration of MDA was significantly higher in preeclampsia as compared to non-pregnant women [19]. The mean plasma and placental levels of malondialdehyde were significantly higher in the pre-eclampsia

compared with uncomplicated pregnancy [11]. The serum MDA levels were raised significantly in women with mild and severe pre-eclampsia in comparison to normal controls [20]. There was a significant increase in erythrocyte MDA levels, in patients with pregnancy-induced hypertension when compared to controls [21].The findings in the present study confirm the elevated levels of MDA in pre-eclampsia.

The decreased total antioxidant capacity (TAC) observed during pregnancy is an indication of increased consumption and/or decreased production of antioxidants. While increased consumption of antioxidants is due to increased scavenging of oxidants, decreased production is perhaps an adaptive mechanism evolved during evolutionary process to facilitate the recruitment of ROS in several signaling cascades of developmental process. The drastic fall in the levels of TAC seen in preeclampsia is possibly indicative of a critical limit of TAC for a normal pregnancy; deviation from which can cause harmful disequilibrium. Studies have shown that the levels of TAC were low before 30 weeks of gestation as compared to non-pregnant women [22]. Highly significant decline in TAC and marked decrease in levels of non-enzymatic antioxidants (vitamins E, A and C) were observed in pregnant women as compared to non-pregnant women [7, 8]. TAC in pre-eclamptic women was lower than those in normal pregnant and non-pregnant women [19]. Serum total anti-oxidant activity and glutathione levels were decreased in women with preeclampsia compared to the normotensive pregnant women [23]. The finding of our study supports a similar change in TAC levels.

We found increased activity of superoxide dismutase in this study. Earlier studies observed significantly high SOD in third trimester than in non-pregnant women [22]. Also the activity of SOD in women with pregnancy-induced hypertension was high when compared to normal pregnant women [21,22]. The level of SOD activity correlated with degree of pre-eclampsia [24].in contrast, some studies showed significantly lower levels of superoxide dismutase in pre-eclampsia as compared to normal pregnancy [11,23]. Therefore, SOD can perhaps be better understood as a modulator of oxidative stress than as an antioxidant per se. It converts superoxide radical into hydrogen peroxide. Hence, increased levels of SOD observed during pregnancy may reflect the process of fine modulation.

In the present study, plasma uric acid levels were also elevated during normal pregnancy and further augmentation was found in pre-eclamsia. Uric acid is the end product of purine metabolism. An excessive catabolism of nucleic acids and/or an inability to adequately excrete uric acid can lead to increased plasma uric acid levels. Increased uric acid levels seen in preeclampsia can be attributed to placental ischemia and damage and possibly to consequent increase in ROS production causing excessive nucleotide catabolism. It is remarkable that uric acid, itself a product of oxidative stress is a powerful antioxidant and the system is using this to its advantage to ameliorate the oxidative stress. There are research studies which suggest an

alteration in renal function in pregnancy resulting in decreased net re-absorption in early pregnancy and increased net tubular re-absorption of uric acid as pregnancy progressed [25]. Uric acid values were significantly increased in pre-eclamptic women and diabetic pre-eclamptic as compared to controls [26].

A recent study showed that oxidative stress markers like MDA, $PGF_{2\alpha}$ were significantly higher and SOD, TAC were significantly lower in pre-eclamptic women, as compared to healthy pregnant women [27].

LIMITATIONS OF THE STUDY

More accurate methods of estimation like high-performance liquid chromatography (HPLC) with UV detection for Malondialdehyde, Trolox Equivalent Antioxidant Capacity (TEAC) assay for Total Antioxidant Capacity and Single Cell Gel Electrophoresis (SCGE) or Comet assay to assess DNA strand breaks, can perhaps give more reliable information. A study involving a larger sample can also be undertaken. Longitudinal study can be done in different trimesters of pregnancy.

Conflict of interest: the authors declare no conflict of interest.

CONCLUSION

Pregnancy is considered as a physiologically stressful state and is reflected by increased levels of oxidative stress markers. The role of ROS as a regulatory mediator of physiological responses and as an important component of several signaling cascades is well documented. Several of these signaling pathways are involved in developmental pathways. Thus it is possible that increased production of ROS in pregnancy is not just a reflection of increased stress but an evolutionarily conserved trait because of its necessary role in pregnancy. In this connection, it is noteworthy, that in early pregnancy the excretion of uric acid, a powerful antioxidant, is increased leading to reduced plasma levels. This is perhaps to tilt the redox homeostasis in favor of ROS so as to allow their unhindered participation in the developmental process which is completed by the end of first trimester.

However, too extreme a shift in redox homeostasis to excessive ROS, especially in late pregnancy causes ROS-induced cell damage and pre-eclampsia. These facts lead to the logical conclusion that there is a physiological upper limit to ROS levels for each stage of pregnancy which is tightly regulated. Objective quantification of these critical levels of ROS needs further research. Other physiological stress models, for example exercise, can be studied for a better understanding of physiological versus pathological role of ROS.

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