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## **Original Article**

# **Total Antioxidant Activity in Normal Pregnancy**

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Objective: Pregnancy is a state, which is more prone for oxidative stress. Various studies report development of a strong defence mechanisms against free radical damage, as the pregnancy progresses. Aim of our study is to assess the antioxidant status by measuring the total antioxidant activity. Methods: Total antioxidant activity was assayed by Koracevic' et al's method, with the plasma of twenty five pregnant women (with normal blood pressure) as test group and twenty five age matched non-pregnant women as control group. All complicated pregnancies are excluded from the study. Results: Highly significant decline (P< 0.001) in antioxidant activity was observed in pregnant women with a value of  $1.40 \pm 0.25$  mmol/l, as compared to controls,  $1.63 \pm 0.21$  mmol/l. Conclusion: Reduction in total antioxidant activity could be due to the fall in individual antioxidant levels. But several studies report an elevated enzymatic and non-enzymatic antioxidants during pregnancy. Any way total antioxidant activity is not a simple sum of individual antioxidants, but the dynamic equilibrium & cooperation between them. So inspite the rise in individual antioxidants, total antioxidant activity may be low. Further studies need to be done with antioxidant activity as a marker of complicated pregnancies like pregnancy induced hypertension.

Key Words: Pregnancy, Antioxidant activity, Oxidative stress



#### **Introduction:**

Pregnancy is a physiological condition in which women are more prone to oxidative stress, which results due to an imbalance between the prooxidant-antioxidant levels. [1] Oxidative stress results because of the presence of placenta which is rich in mitochondria. Fully developed placenta consumes about 1% of the basal metabolic rate of the pregnant woman. It is also highly vascular and is exposed to high maternal oxygen partial pressure. Pregnancy is characterized by increased basal oxygen consumption and changes in energy substrate use by different organs.

These features support the generation of super oxide, as 5% of all electrons in the mitochondrial electron transport chain leak out of the mitochondria.[2] Nitric oxide (NO), which is locally produced by the placenta and along with other reactive nitrogen species, contributes to oxidative stress [3]. Various protective mechanisms develop against free radical generation and damage during pregnancy but the degree of oxidative stress depends on the balance between the defense mechanisms and the free radical producing mechanisms. There are several

studies regarding various individual antioxidants, but there are a few studies regarding the total antioxidant activity (AOA) in pregnancy. Aim of our study is to assess the antioxidant status by measuring total AOA.

### **Methodology:**

The study was conducted in Mangalore city during the year 2006. Twenty five normal pregnant women in the age group 18-39 yrs, who came to Chirashree hospital for their routine check up in their third trimester were selected for the study. Diagnosis was made on clinical and ultrasonography findings. Pre-pregnancy BMI was in the range of 19.8-24.4kg/m<sup>2</sup>. Elderly primigravidas, gestational diabetics, chronic hypertensives, multiple gestations, preeclamptics and eclamptics were excluded from the study. Age matched nonpregnant women were taken as controls. Subjects in our study were nonsmokers, nonalcoholics and not suffering from any illnesses. Informed consent was taken from all the subjects and the study was approved by the institutional ethical committee. Demographic profile of the subjects is shown in Table I.

Table I : Demographic profile of non pregnant & normal pregnant ladies				
	Non-pregnant (n=25)	Normal pregnant (n=25)		
Age (yrs)	$24.3 \pm 4.2$	$25.5 \pm 3.3$		
Gestational age at Sampling (wk)	-	34.3 ±0.8		
Parity	-	2 ±1		
BMI at sampling(kg/m <sup>2</sup> )	21.2 ±1.2	20.3 ±1.9		
n = number of subjects				

One ml of venous blood was collected in EDTA bottles using disposable syringes, plasma was separated. Total AOA was determined by Koracevic' et al's method[4]. The assay measures the capacity of the serum to inhibit the production of thiobarbituric acid reactive substances (TBARS) from sodium benzoate, under the influence of the oxygen free radicals derived from Fenton's reaction. The reaction was measured spectrophotometrically at 532 nm. Antioxidants from the added sample cause suppression of the production of TBARS and the inhibition of colour development is defined as AOA.

## **Statistical Analysis**

Statistical analysis was done by using paired 't' test and data is presented as mean  $\pm$  standard deviation.

### Results

We observed a significant decline in total AOA in pregnant ladies  $(1.40\pm0.25 \text{ mmol/L})$  as compared to non-pregnant ladies  $(1.63\pm0.20 \text{ mmol/L})$ . Blood pressure and AOA of all the subjects are shown in Table II. A positive correlation was observed between diastolic blood pressure and AOA in pregnant women, but it was found to be statistically insignificant.

Table II: Comparison of AOA values of subjects					
	SBP (mmHg)	DBP (mmHg)	AOA (mmol/L)		
Pregnant with normal BP (n=25)	$120.32 \pm 10.04$	$72.04 \pm 6.54$	1.40±0.25 ***		
Non pregnant ladies (n=25)	118 ±4.24	70 ±8.24	1.63 ±0.20		
n= number of subjects; ***= P< 0.001					



## **Discussion:**

We have found a significant reduction in total AOA in pregnancy (P< 0.001). Decreased AOA, is indicative of a disturbance in the antioxidant system, which could be due to diminished individual antioxidants. When we go through previous literature, we come across inconclusive reports.

When we reviewed literatures for enzymatic antioxidants, super oxide dismutase (SOD) activity in erythrocytes and plasma thiol levels were found to be lower and ceruloplasmin levels were found to be higher during pregnancy than in nonpregnant women, suggesting a diminished antioxidant defense.[5,6] We also searched for articles on nonenzymatic antioxidant levels in pregnancy. We found a report suggesting diminished plasma ascorbic acid concentrations in normal pregnancy.[7] Reduction in nonenzymatic antioxidants (GSH, vitamins A, E, C) during normal pregnancy is also reported.[8] Overall we find reduction in individual antioxidant levels in normal pregnancy. With this theory, decreased AOA in our study can be attributed to the decline in various individual antioxidants during pregnancy.

But we found some reports which are contradictory to our result. We could find some articles which suggest aggressive defense mechanisms against free radical damage as pregnancy progresses. Placental homogenates and syncytiotrophoblastic brush border preparations were analyzed from interrupted pregnancies, early, at midgestation and at term. These reports suggest progressive increase antioxidants like bilirubin and glutathione as well as in SOD, catalase and glutathione peroxidase and reductase levels.[9-11] Glutathione peroxidase in RBCs and platelets and extra cellular SOD activity has also been reported to be elevated.[12,13] In conclusion, the body tries to fight the prooxidants and counteract oxidative stress in normal pregnancy through induction of enzymes (i.e., catalase, SOD, glutathione peroxidase, transferase and reductase, glucose 6phosphate dehydrogenase) as well as through nonenzymatic free radical scavengers (i.e., vitamins C and E, uric acid, protein thiols).[14-22]

With this theory we cannot explain diminution in the AOA observed in our study but the total antioxidative capacity is not just a sum of the activities of the various antioxidants but the cooperation of the antioxidants in human serum that provides greater protection against attacks by free radicals. It is the dynamic equilibrium between various antioxidants. So even though individual antioxidants rise during pregnancy, net result may be a lower antioxidative capacity.

However, pregnancy is a state where this adaptation and equilibrium are easily disrupted and result in various complications like hypertensive, diabetic disorder of pregnancy etc. Based on our study results, we can expect a marked diminution of AOA in preeclampsia and eclampsia. Our study results are supporting, the reduced

essential antioxidants observed in preeclampsia.[23] Is this decline in AOA, a result of complicated pregnancy? Or can it be used as a marker of complicated pregnancies? Further studies need to be done regarding using AOA as a marker for predicting complicated pregnancies.

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