

## **Plasma adenosine deaminase activity and antioxidant status in preeclampsia compared to healthy pregnant and nonpregnant women.**

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### **Abstract**

**Preeclampsia is a multisystemic disorder specific to human pregnancy. It is common and major complication, characterized by hypertension, proteinuria and oedema. This clinical condition adversely affects the mother and the foetus. The causative factors remain poorly understood. Oxidative stress is implicated in preeclampsia which could provide the linkage between decreased placental perfusion and the maternal syndrome.**

**Pregnancy has also been associated with depressed cell mediated immunity. The increase in activity of adenosine deaminase (ADA) in preeclamptic women compared to normotensive pregnant women is reported in earlier studies. The main objective of the present study was to correlate the adenosine deaminase activity with the antioxidant status in preeclamptic patients.**

**The study groups aged between 20 – 35 years included 20 normotensive primigravida women and 20 primigravida diagnosed as preeclampsia in their last trimester. The control group included 20 healthy nonpregnant women volunteers of the same age group. The diagnosis of preeclampsia was made by a gynecologist at Lady Goshen Hospital. The adenosine deaminase activity and antioxidant status in the form of superoxide dismutase (SOD), glutathione (GSH) and total antioxidant activity (TAA) were measured. The extent of oxidative stress was estimated by measuring plasma malondialdehyde (MDA) levels.**

**The comparison of MDA between controls and preeclamptic patients showed significance (p 0.000). The total antioxidant activity (TAA) between the controls and preeclamptic patients showed significance (p 0.000). However, ADA activity did not show statistical significance among the different study groups. The ADA activity may not be significant due to other systemic causes such as anemia and malnutrition.**

**Key words:** *Adenosine deaminase, oxidative stress, antioxidants.*

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### **Introduction**

Preeclampsia occurs in 3% to 5% of pregnancies. It is also a major cause of maternal mortality (15% to 20%) in developed countries and a leading cause of preterm birth and intrauterine growth retardation [1,2].

There is substantial evidence to suggest that the endothelial cell injury and altered endothelial cell function may play a role in pathogenesis of preeclampsia [3,4]. The vascular endothelium is the target for the disease process involved in preeclampsia [5]. Maternal vascular endothelial dysfunction may be the cause of altered vascular reactivity, vasospasm and platelet aggregation [6].

Lipid peroxidation and antioxidant status has been implicated in the aetiology of preeclampsia [7,8,9]. Oxidative stress is a component of preeclampsia, which could provide the linkage between decreased placental perfusion and the maternal syndrome [10]. Many reports suggest that lipid peroxidation products, primarily measured as thiobarbituric acid reactive substances which include malondialdehyde (MDA) are increased in plasma / sera of women with preeclampsia [7,8].

Increase in plasma adenosine deaminase (ADA) activity in preeclamptic women compared to the normotensive pregnant women has been reported [11]. Serum total antioxidant activity (TAA), erythrocytic superoxide dismutase (SOD) activity and glutathione levels are decreased

in women with preeclampsia compared to the normotensive pregnant women [12-14]. However, no correlation between ADA activity and antioxidant status has been reported and this will be the main objective of the present study.

## Subjects and Methods

The research design included three study groups as follows.

Group I (Control) - 20 healthy nonpregnant women

Group II (Normal Pregnant) - 20 normotensive Primi gravida women

Group III (Preeclamptic)- 20 primigravida women with preeclampsia

All the subjects included were aged between 20 - 35 years. The control group comprised of healthy volunteers of the same age group. The subjects admitted to obstetric wards of the Lady Goshen Hospital of Mangalore were selected. The diagnosis of preeclampsia was made by a gynecologist at Lady Goshen Hospital. An informed consent was obtained from all the subjects enrolled for the study. The study was approved by the Institutional Human Ethics Committee.

*Inclusion criteria:* Normotensive primigravida women and preeclamptic primigravida in their last trimester

*Exclusion criteria:* Multigravida, pregnant women with anemia and gestational DM were excluded.

### Sample Collection

Blood was collected from Group II and Group III subjects between 33-36 weeks of pregnancy. 5ml of venous blood was collected randomly from the antecubital vein under aseptic precautions from each subject. 2.5 ml of whole blood was collected in EDTA bottles and 2.5 ml whole blood was collected in plain bottles. The sample was then subjected to centrifugation for 3000g for 10 minutes within three hours of collection. Plasma collected carefully was used for the assay of lipid peroxidation and adenosine deaminase. Serum separated from whole blood collected in plain bottles was used for the assay of total antioxidant activity. Remaining whole blood was mixed with 0.9% saline and centrifuged. Supernatant was removed and this process was repeated three times to prepare RBC suspension which was used for assay of SOD and reduced glutathione.

*Plasma MDA* - was measured using the modified method of Satoh [15-17]. Malondialdehyde a secondary product of lipid peroxidation, reacts with thiobarbituric acid (TBA) in acidic medium to give a pink coloured pigment

at 97°C at pH 2-3. The pink colour was extracted with butanol and the absorbance read at 535 nm.

*Adenosine deaminase* - was measured by using Giusti's method [18]. Ammonia is liberated by the action of adenosine deaminase on adenosine. Ammonia forms an intense blue indophenol with phenol nitroprusside solution and alkaline sodium hypochlorite. The reaction catalyzed by ADA is stopped at the end of the incubation period by the addition of the phenol nitroprusside. The intensity of the blue colour is directly proportional to the amount of ammonia liberated and was measured spectrophotometrically at 620nm.

*Total antioxidant activity* – was measured by the method of Koracevic *et al* [19]. The assay measured the capacity of the serum to inhibit the production of thiobarbituric acid reactive substances (TBARS) from sodium benzoate under the influence of free oxygen radicals derived from Fenton's reaction. The pink colour developed was measured spectrophotometrically and the inhibition of color development defined as AOA.

*SOD* – The method of Beauchamp and Fridovich was followed [20]. Illumination of riboflavin in the presence of O<sub>2</sub> and electron donor like methionine or EDTA generates superoxide anions (O<sub>2</sub><sup>•-</sup>) and this has been the basis for assay of SOD. The reduction of nitrobluetetrazolium (NBT) by O<sub>2</sub><sup>•-</sup> to blue coloured formazan was followed at 560nm.

*Glutathione* – was determined by the method of Beutler *et al* [21]. This is based upon the development of a relatively stable yellow color when 5, 5' – dithiobis dinitro benzoic acid is added to sulphhydryl compounds. The yellow color was measured at 412nm.

### Statistical analysis

The data was analysed using SPSS software version 15.0. For the comparison of values between the groups, one way analysis of variance was used when the data was normally distributed. Kruskal Wallis test was used for skewed data. For the correlation, Pearson's correlation coefficient was used.

## Results

A highly significant increase in MDA value (p 0.000) in normal pregnant & preeclampsia in comparison with the controls was observed with the mean value of 113.32 & 133.07nmol/dl respectively (Table I). However, there was no statistical significance between the normal pregnant and preeclampsia. Statistically significant reduction in TAA & GSH was observed in preeclampsia when compared to normal pregnant with the mean value of

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1.4020 & 1.6150 mmoles/L and 36.49 & 43.41 mg/dl (Table II) with the p value of 0.039 & p 0.048 respectively. A significant reduction in SOD (7442.21 Units/gmHb & 5264.98 Units/gmHb), TAA (1.7700 mmoles/L and 1.4020 mmoles/L) and GSH (50.01 mg/dl and 36.49 mg/dl) in preeclampsia was observed when compared to

controls. A weakly positive association was observed with ADA Vs glutathione & TAA (Figures 1,2). However, ADA did not show any statistical significance in either of the groups. Also, there was no correlation between plasma adenosine activity and MDA or the antioxidant enzyme SOD.

**Table I: Adenosine deaminase and antioxidant status in preeclampsia**

No.	Parameter	Control (n =20) (Mean ± SD)	Normal Pregnant (n=20) (Mean ± SD)	Pre eclamptic (n=20) (Mean ± SD)
1	Age	24.85 ± 2.13	24.50 ± 4.45	28.05±4.28
2	MDA nmoles/dl	62.26 ± 24.63	113.32 ± 8.97 *	133.07±8.57 *
3	TAA mmoles/L	1.7700 ± .25	1.6150 ± .28	1.4020 ±26 **†
4	ADA Units/L	17.55 ± 6.19	21.48 ± 8.09	22.79 ±8.04 *
5	SOD Units/gmHb	7442.21 ± 1489.96	6136.07 ± 15638.76	526498±2202.16
6	GSH mg/dl	50.01 ± 8.26	43.41 ± 10.98 *	36.49±7.44†

\*As compared to control at 0.001 level of significance

† As compared to preeclampsia at 0.05 level of significance

MDA – malondialdehyde; TAA – total antioxidant activity; ADA –adenosine deaminase; SOD - superoxide dismutase  
GSH – glutathione

**Table II: Significance levels of the parameters between the control, normal pregnant & preeclampsia subjects**

Parameter	C Vs NP	C Vs PE	NP Vs PE
MDA	.000♦♦♦	.000***	.238
TAA	.170	.000***	.039*
ADA	.231	.078	.844
SOD	.061	.001***	.278
GSH	.062	.000***	.048 *

C – Control; NP – Normal pregnant; PE – Pre eclampsia

♦ - C Vs NP; \* - C Vs PE ;

\* NP Vs PE ;

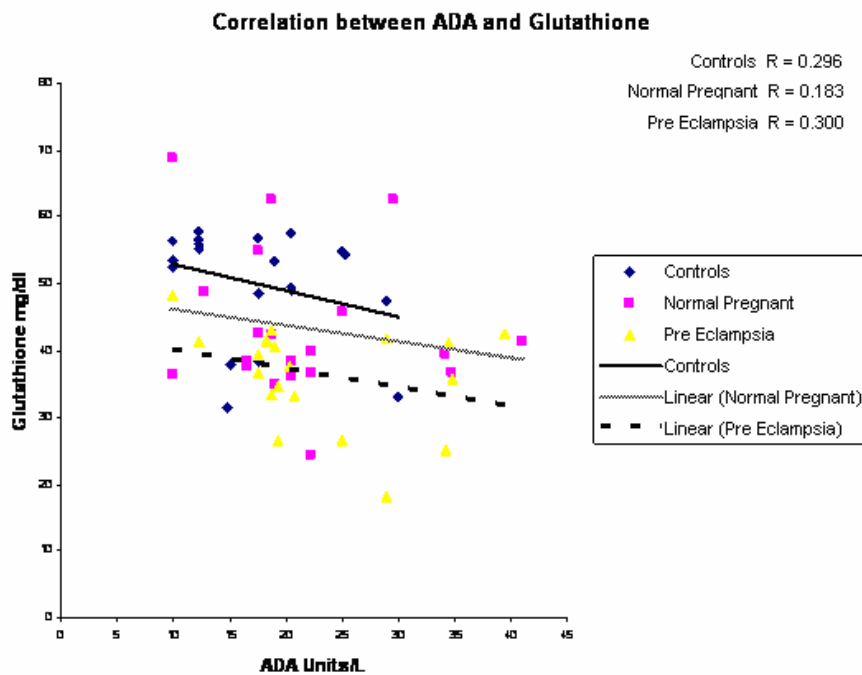


Figure 1

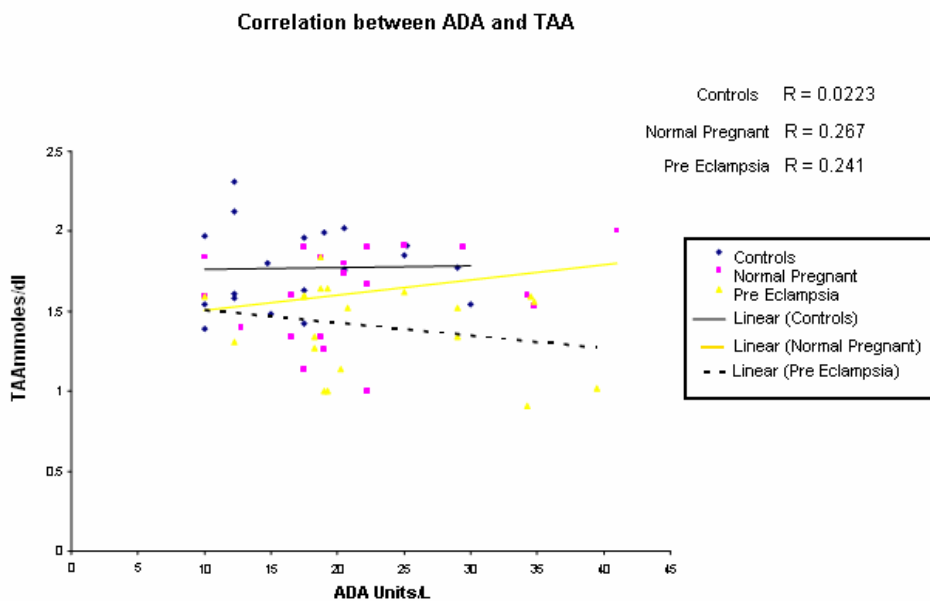


Figure-2

**Discussion**

Preeclampsia is characterized by endothelial dysfunction and alterations of immune response may be involved in the pathogenesis of this disease. Pregnancy has been associated with depressed cell mediated immunity [22]. Oxidative stress may be an important factor in the pathogenesis of preeclampsia and the antioxidant status in preeclampsia is complex [23].The possible role of cell

mediated immunity in preeclampsia has been evaluated by assessing the adenosine deaminase activity [11].

This enzyme is essential for the differentiation of the lymphoid cells and has been used for monitoring several diseases in which immunity is altered [24].

The present study demonstrated a significant increase in plasma MDA in preeclampsia as compared to normal

pregnant women. This finding is in agreement with other studies [14,25,26]. Yoneyama *et al* [27] have also reported increased plasma MDA in preeclampsia which was positively correlated to ADA activity. On the other hand, Jeronimo *et al* have reported decrease in plasma ADA in normal pregnant (24). On the contrary, the present study reports non significant ADA levels without any correlation of MDA with ADA activity suggesting the absence of T-cell activation.

An important finding of the study is the statistically non significant difference in the ADA levels. Several studies have reported increase in plasma ADA in preeclampsia which in turn may be responsible for maintenance of immune response [28, 29]. Karabulet *et al* have opined that increased ADA as a marker of immunological disorder may be related to the pathogenesis of the disease [28].

The antioxidant status is reflected by a decrease in RBC SOD and GSH in preeclampsia and normal pregnant. These findings are in line with previous studies [14, 30,31,32]. There was no correlation between lipid peroxidation and antioxidant enzymes. This is in agreement with the report of Bayhan *et al* [14].

That oxidative stress is the principal causative factor, is reflected by increase in MDA and decrease in TAA activity. Significant decrease in TAA is observed not only in preeclampsia but also in normal pregnant. A 12.5 fold decrease was observed in preeclampsia when compared to normal pregnancy. This finding is consistent with the report of Davidge *et al* [33] and Harma *et al* [34].

Current theory holds that oxidative stress is an imbalance between maternal prooxidants and antioxidants. From the results it can be concluded that impaired antioxidant activity and the reduction of antioxidants could be the possible cause for the increased lipid peroxidation observed which may cause damage to vascular endothelium resulting in clinical symptoms of preeclampsia. Administration of antioxidants in early pregnancy may help in preventing the complication resulting in preeclampsia and may be a fruitful therapeutic strategy. One of the limitation of the study was the assessment of the antioxidant status was done only during last trimester and not followed at the time of delivery. Further studies on the mechanism of ADA regulation may provide information on its added role in the pathogenesis of preeclampsia.

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