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Research Article

STUDY OF SERUM ASCORBIC ACID LEVEL IN PRE-ECLAMPSIA

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ABSTRACT

The present study suggests that in preeclampsia there is an increase in the lipid peroxidation products and leads to a decrease in the plasma antioxidants except uric acid and changes in the lipid profile levels, contributing to the pathogenesis of pre-eclampsia. With this context, the present study was undertaken to determine the change in plasma antioxidant level in women with preeclampsia.

Objectives: To measure the level of ascorbic acid in pre-eclampsia in comparison with normal pregnancy.

Materials and Methods: Cross sectional study was conducted consisting of 30 pre-eclamptic and 30 healthy pregnant women. Fasting venous blood samples were collected during antepartum period and plasma levels of ascorbic acid were estimated in both the groups.

Results: In the pre-eclamptic group plasma antioxidant ascorbic acid was significantly decreased

Conclusion: Lipid peroxidation is important factors in the pathogenesis of pre-eclampsia. The plasma antioxidants are excessively utilized to counteract the cellular changes mediated by free radicals in pre-eclampsia.

Keywords: Pre-eclampsia, Antioxidants, Ascorbic acid, Lipid Peroxidation, Oxidative stress.

INTRODUCTION

Pregnancy is a physiological stress in which many changes occur in the milieu interior of the body, more and more stress is being laid on the biochemical changes, which occur in the blood during the normal pregnancy and becomes exaggerated in complications of pregnancy like pre-eclampsia[1]. Pre-eclampsia is defined as a pregnancy-specific syndrome observed after the 20th week of pregnancy with systolic blood pressure of ≥ 140 mm of Hg or diastolic blood pressure of ≥90 mm of Hg accompanied by significant proteinuria (i.e., urinary excretion of ≥ 0.3 g protein in a 24-h specimen).In women with pre-eclampsia, blood pressure usually returns to baseline within days to weeks after delivery[2].

Pre-eclampsia is a complex multisystem disorder seen exclusively in the human species. Worldwide, it is a leading cause of maternal and fetal morbidity and mortality[3]. Pre-eclampsia is a hypertensive disorder which develops in late pregnancy and is usually associated with placental hypoxia and dysfunction[6]. Various factors are implicated in the pathogenesis of pre-eclampsia, including genetic, immune, vascular and oxidative stress[7]. Pre-eclampsia occurs during second and third trimester of pregnancy and is more common in nulliparous women .Proteinuria is an important sign of pre-eclampsia and Chesley (1985) rightfully concluded that the diagnosis is questionable in its absence[1].

It is well known that oxidative stress increases during normal pregnancy. In healthy pregnancy, it has been reported that plasma lipid hydro peroxides levels are increased and total antioxidant capacity is decreased. More oxidative stress in pre-eclampsia results in lipid peroxides, reactive oxygen species and super oxide anion radicals to cause endothelial injury and dysfunction, platelet and neutrophil activation, increased cytokines, superoxide radical production and endothelial damage in a vicious cycle[4]. An increase in resistance to angiotensin, a predominance of lipid metabolism over glucose utilization and an increased synthesis by the liver of thyroid and steroid-binding proteins, fibrinogen and other proteins are characteristic of pregnancy. Plasma lipids and lipoproteins undergo both quantitative and qualitative changes during pregnancy[5]. These observations on the effects of oxidative stress in pre-eclampsia have given rise to increased interest in antioxidants, such as vitamin C (Ascorbic acid), vitamin E, Uric acid etc.

Oxidative stress increases during pre-eclampsia and results in increased production of lipid peroxides, reactive oxygen species and superoxide anion radicals to cause endothelial injury and dysfunction, platelet and neutrophil activation[5]. These observations in pre-eclampsia have given rise to increased interest in oxidants and antioxidants. The present study has been undertaken to determine the change in serum level ascorbic acid in women with pre-eclampsia.

MATERIALS AND METHODS

The study was carried out in 30 pre-eclampsia primi patient and 30 normotensive primi pregnant controls who attended the outpatient and inpatient departments of Kempegowda Institute of Medical Sciences, Bangalore during the year 2011-12. The institutional ethical committee approved the study protocol.

Inclusion criteria:

Cases of pre-eclampsia primi patients in the age group of 18 to 30 years and with gestation age more than 20 weeks. Controls consisted of normotensive primi pregnant women in the age group of 18 to 30 years and more than 20 weeks of gestation.

Exclusion criteria:

Elderly primi gravida subjects, gestational diabetes, chronic hypertension, multiple gestation, those with family history of pre-eclampsia, acute and chronic infections, renal diseases, liver diseases, endocrine disorders, smokers, alcoholics and with history of multivitamin intake.

Informed written consent was taken from patients and controls. A pre-structured and pre-tested proforma was used to collect the data. Baseline data including age and BMI, detailed medical history, clinical examinations and relevant investigations were included as part of the methodology.

Sample collection: 5 ml plain venous blood sample after overnight fasting was obtained by venepuncture from both cases and controls. This was followed by centrifugation and then sample was processed immediately. Estimations of serum Ascorbic acid were performed using the serum by 2, 4 – Dinitrophenyl hydrazine Method [14].

Principle: Ascorbic acid is oxidized by copper to form dehydroascorbic acid and diketogulonic acid. These products are treated with 2, 4, DNPH to form the derivative bis-2, 4 dinitrophenylhydrazone. This compound, in strong sulfuric acid, undergoes rearrangement to form a product with an absorption that is measured at 520nm. The reaction is run in the presence of thiourea to provide a mildly reducing medium, which helps to prevent interference from non-ascorbic acid chromogen.

Reagents:

1. Trichloroacetic acid (TCA) 10%: 10 gm of TCA dissolved in distilled water & volume made: up to 100ml.
2. 2, 4 dinitrophenyl hydrazine/thiourea/ copper sulphate (DTC) solution: 0.4 gm thiourea, 0.05gm $CuSO_4$ 5H2O and 3.0 gm DNPH were added to 9 N $H_2SO_4$and the volume was made up to 100 ml.
3. 65% H_2SO_4: 65ml of con H_2SO_4 is dissolved in 35 ml distilled water.
4. Stock standard: 100 mg ascorbic acid is dissolved in 100 ml of 5% TCA.
5. Working standard: 1 ml of stock standard is dissolved in 100 ml of 5% TCA.

Procedure:

Test: 1.0 ml of serum was taken in a centrifuge tube, 1.0 ml of ice cooled 10% TCA was added, mixed thoroughly and centrifuged for 20 minutes. From this 0.5 ml of supernatant was taken in a test tube marked as T.

Standard: 0.5 ml of working standard was taken in a test tube marked as S.

Blank: 0.5 ml of 5% TCA was taken in test tube marked as B.

To all the test tubes 0.1 ml of DTC reagent was added and incubated at 37 °C for three hours.

Then 0.75 ml of ice cooled 65% H_2SO_4 was added to all the test tubes and mixed well. Test tubes were allowed to stand for 30 minutes at room temperature. The absorbance was read at 520 nm against blank.

Calculation

Concentration of serum ascorbic acid =

$$\frac{\text{Optical Density of Test}}{\text{Optical Density of Standard.}} \times \frac{\text{Concentration. of Standard}}{\text{Volume of Test}} \times 100$$

$$\frac{\text{Optical Density of Test}}{\text{Optical Density of Std.}} \times 2 = \text{.....mg/dl}$$

Reference range: Normal serum level: 0.4-1.5 mg/dl

RESULTS

The present study is undertaken to evaluate the significance of serum ascorbic acid level in pre-eclampsia.30 pre-eclampsia cases were considered for the study. 30 ages matched normotensive primi pregnant were chosen as controls. The distribution of the study samples according to the age is given in Table 1. The cases and controls are divided into 3 groups (≤ 20years, 21-24yrs, ≥ 25yrs). Maximum numbers of cases are in the age group of ≥ 25yrs (53.34%) and maximum numbers of controls are in the age group of 21-24yrs (63.33%).

Distribution of study sample according to gestational age group

The distribution of the study samples according to the gestational age is graphically represented in figure 1. The cases and controls are divided into 2 groups (22-28 weeks and 29-34 weeks). Maximum numbers of cases are in the gestational age group of 29-34 weeks (56.67%) and maximum numbers of controls are in the gestational age group of 22-28 weeks (53.33%).

Comparison of Blood Pressure between cases and controls

Comparison of Blood Pressure between cases and controls are shown in the Table 2 and graphically represented in figure 2, respectively. The mean value of systolic blood pressure among cases as compared to controls was statistically significant, (p value < 0.001,t test value 17.02) and mean value of diastolic blood pressure among cases as compared to controls was statistically significant,(p value < 0.001,t test value 28.31).

Comparison of Serum ascorbic acid level between cases and controls

The mean serum ascorbic acid levels are lower among cases as compared to controls was statistically significant (p value < 0.01, t test value 15.28). Distribution of controls and cases according to serum ascorbic acid level is graphically represented in figure 3.

Table 1: Age distribution of cases and controls

Age in years	Cases		Controls	
	Number	%	Number	%
≤ 20	4	13.33	2	6.67
21-24	10	33.33	19	63.33
≥25	16	53.34	9	30.00
Total	30	100.00	30	100

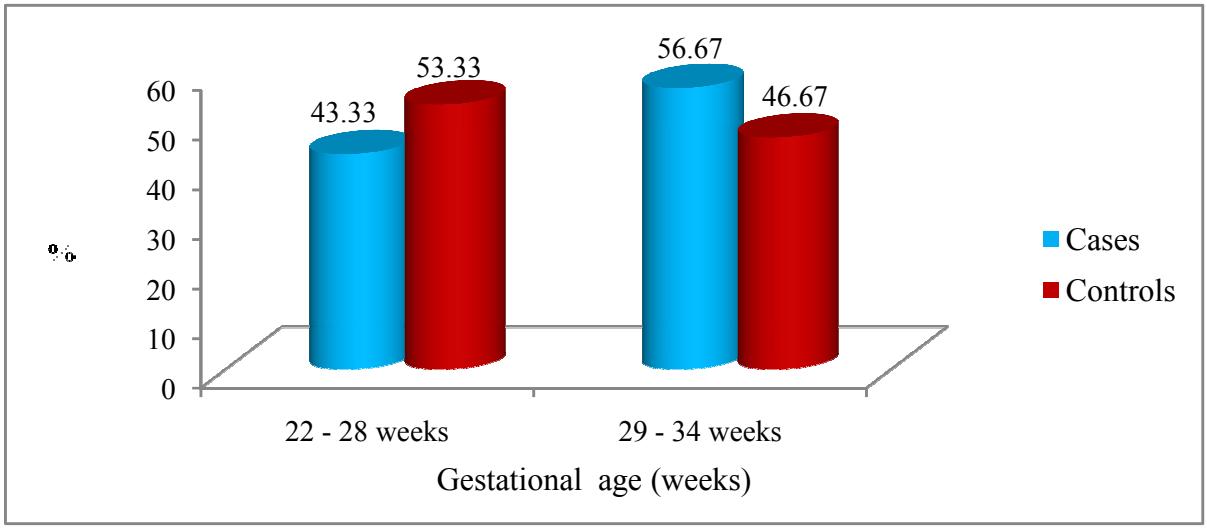

Figure 1: Graph showing the gestational age of Cases and Controls

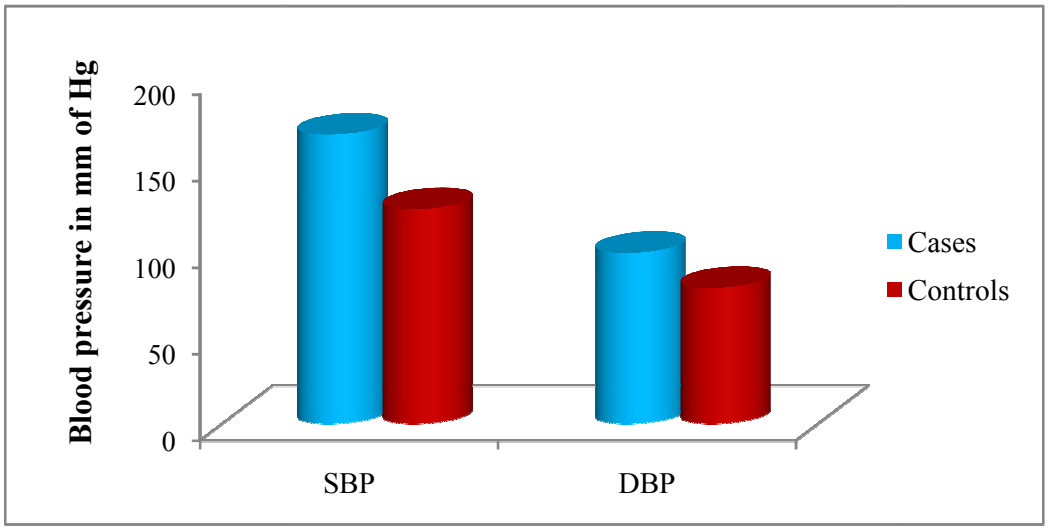

Figure 2: Bar diagram showing the mean blood pressure in both groups

Table 2: Mean Blood Pressure Levels in cases and controls

		Cases	Controls	t test values	P values
BP (mm Hg)	SBP	167.07 ± 12.82	123.93 ± 5.32	17.02	0.001
	DBP	98.67 ± 2.43	78.4 ± 3.08	28.31	0.001

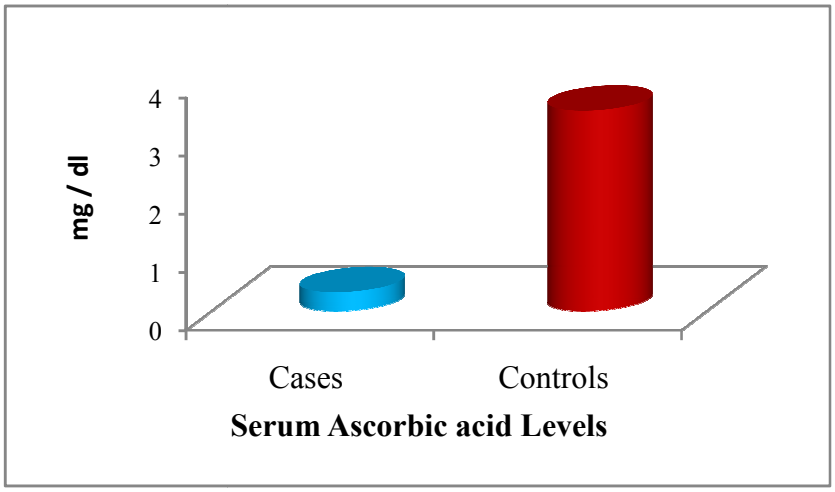

Figure 3: Mean serum ascorbic acid levels in both groups

Table 3: Comparison of Serum ascorbic acid level between cases and controls

Biochemical parameters	Cases	Controls	t test value	p values
S. Ascorbic acid (mg/dl)	0.32 ± 0.06	3.44 ± 1.12	15.28	0.001

DISCUSSION

Preeclampsia remains one of the most serious complications of pregnancy. The pathophysiology of the disease remains poorly understood. The exact cause of preeclampsia remains elusive; placental ischemia, immune maladaptation, genetic factors are probably all involved to some extent. In normal pregnancy the diameter of the spiral arteries increases greatly due to trophoblastic invasion of the spiral arteries in the decidual and myometrial segments of the placental bed, whereas in preeclampsia such physiological adaptation does not occur[16]. Abundant evidence indicates reduced placental perfusion in preeclampsia [17].Implantation is superficial in preeclampsia. In particular, cytotrophoblasts fail to invade the spiral arterioles. As a result, these vessels do not enlarge, severely compromising their ability to deliver maternal blood to the intervillous space. Predisposing to the medical condition[18]. Recent investigations suggest that endothelial cell injury may be the initiator of the pathophysiological events of preeclampsia[12].

Free radicals and other damaging reactive oxygen species, such as the superoxide anion, are produced in oxidative metabolic and physiological processes. Their activity is thought to increase during pregnancy and especially during preeclampsia[13]. Feto-placental unit may be the origin of oxygen free radicals and lipid peroxides[19,20]. Reactive oxygen species can cause cellular damage by oxidizing nucleic acids, proteins and membrane lipids[15]. They may also influence vascular tonicity, either indirectly by inactivating the endothelium derived relaxing factor, which is nitric oxide, and reducing the release of prostacyclin or directly by contracting smooth muscles[21]. Such events establish a cycle ultimately leading to manifestations of preeclampsia[11]. Thus uncontrolled lipid peroxidation may play an important role in the pathophysiology of preeclampsia.

Preeclampsia is associated with an imbalance between the oxidant and antioxidant status. Preeclamptic patients are exposed to increased oxidative stress. Either placental hyper secretion of lipid peroxides or decreased placental antioxidant enzyme activity can lead to endothelial dysfunction. Insufficient antioxidant capacity leads to oxidative stress, and subsequently, oxidative injury may occur in both the maternal and placental compartment[13].

Uncontrolled lipid peroxidation may contribute to various disease processes via disruption of membrane lipids and cell components [11]. Lipid peroxidation of membrane associated fatty acids and cholesterol may alter cell membrane fluidity and permeability, causing cell membrane damage [9]. The byproducts of tissue lipid peroxidation propagate further lipid peroxidation in the same tissue and at sites distal to areas of initial damage [21]. A number of reports indicate that blood levels of lipid peroxidation products are elevated in women with preeclampsia relative to normal pregnancy[8,22]. Further more placental production of lipid peroxides has been demonstrated to be abnormally increased in preeclampsia[19].

The antioxidant vitamin C, have important roles in the defence mechanisms against lipid peroxidation. As a low molecular weight water soluble anti-oxidant, ascorbic acid traps most of the free radicals present in the aqueous phase of the plasma and functions as a first line defence mechanism against free oxygen radicals[23].

In preeclampsia, antioxidant activity is generally but not uniformly low. Plasma ascorbate level decreases gradually throughout normal pregnancy. When Chappell et al compared antioxidant concentrations in high and low risk women, base line concentrations of specific antioxidants were lower (vitamin C), higher (uric acid), or the same (α-tocopherol). This suggests that the measurement of a single antioxidant in a particular biological fluid or tissue at a given point in

pregnancy may not adequately reflect the balance between pro-oxidant and antioxidant forces[24].

Preeclampsia is associated with increased utilization of antioxidants. Several studies have demonstrated decreased plasma levels of vitamin C compared to normal pregnant women[20]. Similarly, the present study observed a significant decrease in plasma levels ($P < 0.001$) of vitamin C in the preeclamptic patients. Vitamin C is the first antioxidant exhausted by oxidative stress[24]. The decrease in plasma antioxidant levels seen in preeclampsia is most probably due to the increased lipid peroxidation[11].

Previous study suggested that vascular endothelial cell dysfunction in pre eclampsia may be caused by uncontrolled lipid peroxidation which overwhelms the protective mechanisms of the antioxidants. Vascular contact with placenta originated circulating peroxidation products may cause dysfunction of the vascular endothelium by promoting peroxidative damage of endothelial cell membranes. Since antioxidant deficiency is a cause of lipid peroxide accumulation, vitamin C therapy may alter the disease process if initiated in early gestation to patients at risk. Further studies are needed to clarify the effectiveness of prophylactic antioxidant therapy in preeclampsia[11].

Thus, in preeclampsia, placental abnormality and the associated metabolic changes cause increased oxidative stress[13].

PIH and related disorders are known to affect the functions of various organs involved in lipid and lipoprotein metabolism. The vascular lesions of PIH and arterial lesions of atherosclerosis share a common pathophysiological pathway which involves lipid metabolism[25].

The interaction of plasma lipids, free radicals, and endothelial cells is hypothesized to be of major importance in the early development of vascular dysfunction in diabetes [10]. Whether such interactions contribute to the pathophysiological mechanisms of preeclampsia warrants further analysis.

CONCLUSION

Serum ascorbic acid levels in pre-eclampsia cases have been evaluated with age and BMI matched controls. The Serum ascorbic acid levels are significantly low in pre-eclampsia patients compared with controls. The present study is consistent with previous studies suggesting that lipid peroxidation appears to be of immense value in understanding the pathogenesis of preeclampsia. In pre-eclamptic patients, antioxidants may be utilized to a greater extent to counteract free radical mediated cellular changes, resulting in the reduction of plasma antioxidant levels.

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