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Research Article

A STUDY ON THE LEVELS OF NITRIC OXIDE AND LIPID PEROXIDES IN PRE-ECLAMPSIA

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ABSTRACT

Hypertensive disorders are the most common medical complications of pregnancy. This contributes significantly to the cause of maternal and perinatal mortality and morbidity. Preeclampsia is a syndrome peculiar to pregnancy characterized clinically by hypertension and proteinuria. The present study compared the levels of serum and urinary nitric oxide (NO), serum and urinary lipid peroxides and total antioxidant status between preeclamptic patients and age and sex matched normal controls. Serum NO concentration in preeclamptic women was significantly higher when compared to normal pregnant women, whereas a significant decrease was observed in urine NO level in preeclamptic woman. There was an increase in the serum total antioxidant capacity and lipid peroxidation product (MDA) in both serum and urine samples of pre-eclamptic women when compared to normal pregnant woman.

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INTRODUCTION

Preeclampsia, a syndrome peculiar to pregnancy is characterized clinically by hypertension and proteinuria¹. In India the incidence of preeclampsia is reported to be 8-10 percent of the pregnancies². Pregnancy-induced hypertension or Preeclampsia (diastolic blood pressure >90 mm Hg) occurring after 20th week of gestation with proteinuria (either ≥ 300 mg protein per day or an urinary protein/creatinine ratio ≥ 30 mg/mmol)³. Hypertensive disorders are the most common medical complications of pregnancy. This contributes significantly to the cause of maternal and perinatal mortality and morbidity⁴. A single blood-pressure reading of 140/90 mm Hg or above is not uncommon in pregnancy and was reported in nearly 40% of pregnant women in one study⁵. Such a finding carries little risk to the mother or fetus. Persistent hypertension is diagnosed if a high reading is found on two occasions at least 4 h apart. The type of hypertension can be further defined on the basis of other clinical signs, particularly proteinuria and abnormalities of coagulation⁶. Hypertension in pregnancy can be classified into two main groups: women who are hypertensive when they become pregnant⁷ and those who become hypertensive for the first time in the second half of pregnancy⁸. Blood pressure generally falls in the first and second trimesters; therefore women with high blood pressure before the 20th week of gestation are assumed to have pre-

existing hypertension. Preeclampsia is the result of an initial placental trigger, which has no adverse effect on the mother, and a maternal systemic reaction that produces the clinical signs and symptoms of the disorder⁹. Preeclampsia occurs only in the presence of a placenta¹⁰. Nitric oxide (NO), a potent endothelial-derived vasodilator, has been shown to modulate peripheral vasodilator tone. Alterations in the L-arginine-NO pathway may modulate the changes in peripheral arterial tone in normal and preeclamptic pregnancy. Sies¹¹ defined "oxidative stress" as "severe disturbance in the prooxidants-antioxidants balance in favor of the former, thus leading to a potential damage to the cells and organs". Lipid peroxidation has also been proposed to play aetiopathological role in various vascular complications of pregnancy, such as intrauterine growth restriction and gestational diabetes. However, this causal relationship between increased lipid peroxidation and preeclampsia is not universally supported. Lipid peroxides are generated when free radicals interact with polyunsaturated fatty acids in the cell membrane and in plasma lipoproteins. This process can become self-perpetuating, leading to a cascade of lipid oxidation. The assessment of serum total thiobarbituric acid-reactive substances (TBARS) provides a measure of total serum lipid peroxidation, an indicator of whole-body free radical activity. The increased lipid peroxidation leads to the consumption of antioxidants. In the present study we are estimating total antioxidant capacity from

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serum sample and Nitric-Oxide, Lipid Peroxidation from both serum and urine samples.

MATERIAL AND METHODS

Source of Data

The study comprises of 34 normal healthy pregnant subjects & 34 cases of preeclampsia aged between 20 to 35 years attending the Department of Obstetrics & Gynecology AJIMS Mangalore from the year 2010-2012. Ethical clearance certificate was obtained from the institutional Ethical Committee.

The diagnosis of preeclampsia was based on the definition of American college of Obstetrics & gynecologists¹².

Inclusion criteria

34 Primigravida with diagnosed preeclampsia according to A.C.O.G with an age between 20 to 35 years in third trimester and 34 normotensive primigravida women with no protienuria from third trimester and without any age systemic or endocrine disorders and age matched with cases.

Exclusion Criteria

Subjects with the history of diabetes, kidney disease, infections, severe anaemia (Hb<6 gm%), history of smoking , history of high blood pressure prior to pregnancy, multigravida & age above 35 yrs will be excluded.

METHODOLOGY

A sample size of 68 consisting of 34 subjects with preeclampsia and 34 subjects without preeclampsia was selected. 5ml of fasting blood sample was collected aseptically from the anticubital vein using plain vacutainers from 34 subjects each with and without preeclampsia. Serum was separated and analysed for the levels of serum nitric oxide, serum MDA, and serum Total antioxidant status. 5ml of urine was collected in a clean, dry & sterile container for estimation of urinary nitric oxide, MDA. Total antioxidant capacity was carried out by Phosphomolybdenum method¹³. Estimation of Nitric-Oxide Level was done by Griess reagent method.

Statistical Analysis

Results are presented as mean ± standard deviation value. Student’s ‘t’ test was used to correlate between total antioxidant level, nitric oxide, MDA in study and control groups. 'p' value of 0.05 or less was considered significant.

RESULTS

Serum Samples

The mean level of serum total antioxidants in normal individuals is 153.74±28.33 and that of Pre-eclampsia patients is 202±37.44 ‘p’ value statistically significant (p<0.0001).The mean level of serum nitric oxide in normal individuals is 49.88±6.78 and that of Pre-eclampsia patients is 66.77±7.54 ‘p’ value statistically significant (p<0.0001).The mean level of serum MDA in normal individuals is 1.34±0.54 and that of Pre-eclampsia patients is 5.35±0.78; ‘p’ value statistically significant (p<0.0001).

Table 1 Comparison of serum parameters in Normal and Pre-eclampsia patients

Parameters	Normal Mean±SD	Pre-eclampsia Mean±SD	‘P’ value
Nitric Oxide (µM/L)	49.88±6.78	66.77±7.54	P<0.0001
TAC (µg/ml)	153.74±28.33	202±37.44	P<0.0001
MDA (µM/L)	1.34±0.54	5.35±0.78	P<0.0001

p<0.05 is statistically significant. Statistical comparison were performed by Student’s ‘t’ test. Data expressed as Mean±SD.

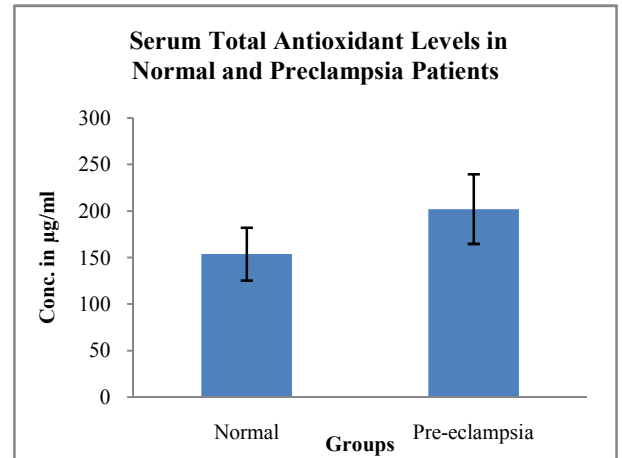


Figure 1 Comparison of serum Total antioxidant levels in normal and preclampsia patients

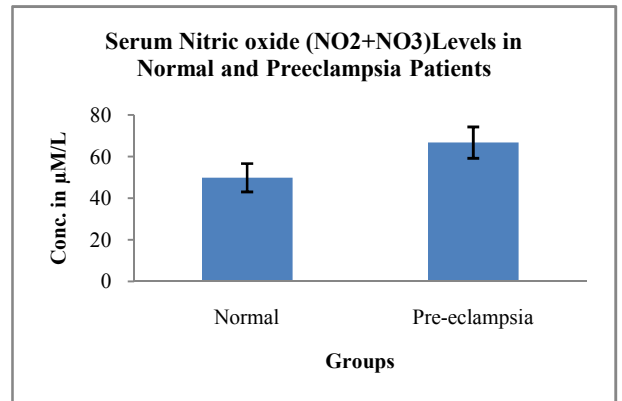


Figure 2 Comparison of serum Nitric oxide (NO₂+NO₃) in normal and preclampsia patients

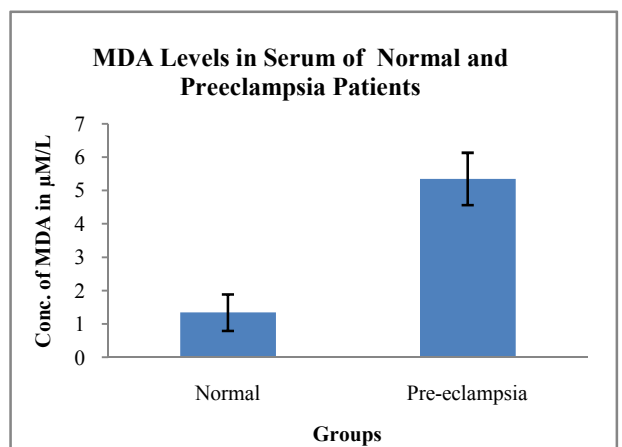


Figure 3 Comparison of serum MDA levels in normal and preclampsia patients

Urine Samples

The mean level of nitric oxide in urine of normal individuals is 645.85 ± 119.84 and that of Preeclampsia patients is 396.08 ± 151.26 'p' value statistically significant ($p < 0.0001$). The mean level of MDA in urine of normal individuals is 1.19 ± 0.62 and that of Preeclampsia patients is 2.47 ± 0.75 'p' value statistically significant ($p < 0.0001$).

Table 2 Comparison of urine parameters in Normal and Preeclampsia patients.

Parameters	Normal Mean \pm SD	Preeclampsia Mean \pm SD	'p' value
Nitric Oxide (μ M/L)	645.85 \pm 119.84	396.08 \pm 151.26	P<0.0001
MDA (μ M/L)	1.19 \pm 0.62	2.47 \pm 0.75	P<0.0001

$p < 0.05$ is statistically significant. Statistical comparison were performed by Student's 't' test. Data expressed as Mean \pm SD.

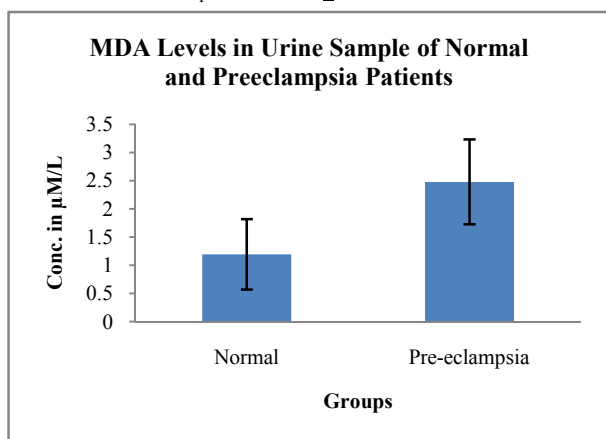


Figure 4 Comparison of urine MDA levels in normal and preeclampsia patients

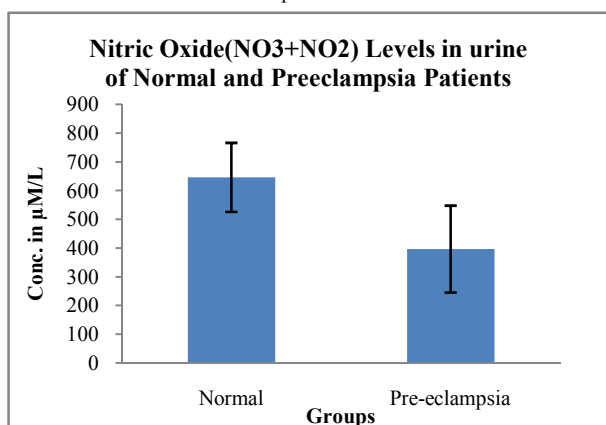


Figure 5 Comparison of urine Nitric oxide ($\text{NO}_3 + \text{NO}_2$) levels in normal and preeclampsia patients

DISCUSSION

There is a complex interaction between free radicals and antioxidants, which modulates the generation of oxidative stress. Oxidative stress occurs when generation of reactive oxygen species (ROS) increases and overwhelms the body's antioxidant defenses. Free radicals are molecules with one or more unpaired electrons in the outer orbit. These include hydroxyl radical, superoxide anion radical, and nitric oxide radical. On the other hand, ROS such as hydrogen peroxide and peroxynitrite do not have unpaired electrons. Human cells have

developed a wide range of antioxidant systems to limit production of ROS, inactivate them, and repair cell damage. ROS are a dual-edged sword. They are key messengers in maintaining physiological functions in the female reproductive tract, however, excessive and unrelenting ROS generation causes various pathologies. Free radicals and other damaging reactive oxygen species, such as the superoxide anions are in oxidative metabolic processes, their activation is thought to increase during pre-eclampsia¹⁴. Foetoplacental unit may be the origin of oxygen free radicals and lipid peroxides^{15, 16}. Recent investigation suggested that endothelial cell injury may be the initiator of the pathophysiological events of pre-eclampsia¹⁷. Either placental hypercorrection 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 compartments¹⁸.

Placental oxidative stress has been proposed as a promoter of lipid peroxidation, and endothelial cell dysfunction associated with preeclampsia¹⁹. Lipid peroxidation has also been proposed to play an etiopathological role in various vascular complications of pregnancy, such as intrauterine growth restriction and gestational diabetes. However, this causal relationship between increased lipid peroxidation and preeclampsia is not universally supported. Some studies have reported that lipid peroxidation is not exacerbated in patients with preeclampsia²⁰.

In this study serum total antioxidant capacity and serum lipid peroxidation product MDA was higher in preeclampsia when compared to normal pregnant woman. Similar results were seen in case of urine MDA levels. The increased MDA levels in preeclampsia is known to be due to increased generation of reactive oxygen species and increased oxygen demand along with reduction in activities of enzymes like superoxide dismutase, glutathione peroxidase and decrease in concentration of antioxidants like Vitamin C and Vitamin E. Reactive oxygen species can cause enhanced lipid peroxidation²¹.

The role of NO in preeclampsia is still uncertain. Lyall *et al.*,¹⁹ found that there was no significant difference in maternal serum nitrite concentrations between a control group and a preeclamptic group. Cameron *et al.*,²⁰ demonstrated that the plasma or urinary nitrate (or nitrite) level was increased during preeclampsia compared to normal gestation. In the present study we observed the serum NO concentrations in preeclamptic women were significantly higher when compared to normal pregnant women, whereas a significant decrease was observed in urine NO in preeclampsia woman. Markedly decreased NO concentrations in urine of preeclamptic women, suggest that NO biosynthesis is decreased in preeclampsia regardless of serum ferritin levels. Because NO is a potent relaxant of vascular smooth muscle, these results suggest that reduced NO production may have an adverse effect on placental hemodynamic function in preeclampsia, and could be involved in the pathogenesis of this important obstetric complication²².

CONCLUSION

Serum total antioxidant capacity and serum lipid peroxidation product MDA was higher in preeclampsia when compared to

normal pregnant woman. Serum NO concentrations in preeclamptic women were significantly higher when compared to normal pregnant women, whereas a significant decrease was observed in urine NO in preeclampsia woman. Further studies are required to substantiate the postulated correlation between the antioxidants activity level and pathophysiology of preeclampsia.

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