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Evaluation of Serum Levels of zinc, magnesium and Malondialdehyde, in the development of Preeclampsia

KEYWORDS

Preeclampsia; zinc, magnesium, MDA

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ABSTRACT **Background:** Zn, and Mg display antioxidant activity and act as peroxynitrite scavengers. The disturbance in the metabolism of these elements may be a contributing factor in the development of certain diseases such as preeclampsia. The aim of the present study was to identify the level of zinc ,magnesium in normal non-pregnant, Group 1 (control), normal pregnant Group II and women with preeclampsia Group III to evaluate the changes in oxidative stress by measuring serum malondiadehyde. **Methods**: A total of 90 female subjects were enrolled in the study in age group of 20-35 years. Blood samples (2-5ml) were collected and serum was separated from each sample. Zn, and Mg were determined by flame atomic absorption spectrophotometry. Lipid peroxidation in each subject was estimated by Thiobarbituric Acid Reactive Substances method (TBARS). **Results:** The serum level of Zn was significantly decreased in patients as compared to controls (66.50 + 0.85, $p<0.001$). We did not find any significant difference in serum Mg level in all three groups (2.01 + 0.02, $p>0.05$). Serum MDA and levels were significantly enhanced in group II and group III subjects as compared to group I and was highest in group III (MDA: 175 +2.69, $p<0.001$. **Conclusions:** The findings suggest that lipid peroxidation along with plasma antioxidants and oxidants may be important factors in the pathogenesis of preeclampsia. These findings have implications for better understanding of preeclampsia and suggest that the oxidative stress has role in pathogenesis of preeclampsia.

Introduction

Preeclampsia is a rapidly progressive condition characterized by high blood pressure, platelet aggregation, swelling of the lower extremities and protein in urine. Sudden weight gain, headaches and changes in vision are important symptoms. It has been a major cause of both maternal and fetal morbidity and mortality.

Oxidative stress is a component of preeclampsia, which could provide the linkage between decreased placental perfusion and the maternal syndrome. Placental Ischemic-reperfusion injury has been implicated in excessive production of reactive oxygen species (ROS), causing release of placental factors that Mediate the inflammatory responses. Therefore preeclampsia has been associated with reduced level of antioxidant enzyme protection causing further placental damage.

The conflicting result of studies evaluating the effects of trace elements and anti-oxidants on clinical outcomes of preeclampsia indicates that the regulatory behavior of trace elements and anti-oxidants need to be validated. Therefore present study was intended to identify the level of trace elements (Zn and Mg) in normal non-pregnant, normal pregnant and women with preeclampsia to know the changes in oxidative stress by measuring the product of lipid peroxidation and serum Malondialdehyde.

Preeclampsia is clinically defined as hypertension and proteinuria with onset following the 20th week of pregnancy [1].. The epidemiology of preeclampsia, being more common in poor women, long ago suggested that nutrients might be involved in the disorder [4]. Some studies have shown that the changes in the levels of blood trace elements in preeclamptic patients may implicate its pathogenesis [5, 4] while others have failed to show an association. Nutritional deficiencies are common during pregnancy and pregnant women in developing countries have been reported to consume diets that are low in minerals and vitamins [5,6]. An inadequate dietary intake before and during pregnancy might be a high risk not only for the mother but also for the foetus. Deficiencies of trace elements such as zinc, copper, selenium and magnesium have been implicated in preeclampsia [8]. Placental and maternal systemic oxidative stress are components of preeclampsia [9] and contribute to a generalized maternal systemic inflammatory activation [10]. Placental ischemia reperfusion injury has been implicated in excessive production of reactive oxygen species (ROS), causing release of placental factors that mediate the inflammatory responses [11].

Zinc deficiency has been associated with preeclampsia since 1980 (12,13,14) including adolescent pregnancies. Some studies reveals that the action of magnesium in a vascular system is present. In fact the production of reactive oxygen species is usually increased in the vasculature of hypertensive patients and the involvement of magnesium could occur through the reduction of inflammation and oxidative stress (15) Malondialdehyde (MDA) is a decomposition product of peroxides polyunsaturated fatty acids and its level has been shown to increase with gestational age (16) MDA is significantly elevated in preeclamptics compared to healthy pregnant women. A significant positive association of MDA with both systolic and diastolic blood pressure was found in preeclamptics (17). Although other compounds (isoprostanes) have been proposed as more reliable indicators of oxidative damage, MDA is still widely used in clinical chemistry laboratories to monitor oxidative stress (18).

Materials and Methods:

The present study was conducted at Department of Biochemistry in association with Obstetrics and Gynecology Department of Princess Esra Hospital, Deccan College of Medical Sciences, Hyderabad. The study was performed after taking approval from the Institutional Review Board (IRB) of Deccan College of Medical Sciences, Hyderabad. All patients enrolled in the study were informed about the study in detail and written informed consent of each patient was received. All the reagents used in the study were of analytical grade and purchased from local distributors.

Study population:

A total of 90 female subjects were enrolled in the study in age group of 20-35 years. All the participants in the study were categorized into three groups each containing 30 subjects. Group I: control group having healthy non-pregnant women, Group II: normal pregnant normotensive women and Group III: diseased subjects having pregnant women with preeclampsia. Preeclampsia was diagnosed by using clinical parameters such as elevated blood pressure (140/90), oedema and proteinuria (by urinary dipstick>1+) as per the minimum specific criteria for the diagnosis of preeclampsia by NHBEP, 2000 report. The control group was selected known to be healthy personnel from the hospital.

Exclusion criteria:

The patients having multiple pregnancies, lactating mothers, smoking and alcoholic habits were excluded from the study. Women with any acute and chronic illnesses (including diabetes, hyperten-

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sion) or taking medications that could potentially affect levels of trace elements were also excluded.

Sample collection and serum separation
Blood samples (2-5ml) were collected from the cubital vein using a sterile syringe into serum collection tubes coated with blood coagulation factors. Tubes containing blood samples were allowed to clot and serum was separated from each sample by centrifuging them for 10 min at 4000 rpm and stored at – 20 deg C until further analysis.

Biochemical analysis of trace elements
Zn, and Mg were determined by flame atomic absorption spectrophotometry as described by Kaneko (1999). Serum level of each trace element was calculated after applying their absorbance values on proper calibration curve prepared from standard solutions. The concentration of trace elements was also correlated with the amount of energy absorbed in each sample.

Estimation of lipid peroxidation
Lipid peroxidation in each subject was estimated by Thiobarbituric Acid Reactive substances method (TBARS). Using this method we evaluated the reactive products of malondialdehyde (MDA) which is the last products of lipid breakdown after oxidative stress. The absorbance was measured at 532 nm using Ultraspec III UY / Visible Spectrophotometer (Plaser et al., 1966).

Statistical analysis
All the results were expressed as mean + standard deviation (SD). Statistical analysis was carried out using Graph Pad Prism (version V) and Microsft Excel 2007. Variance analysis (one-way and two way ANOVA) was performed for multiple comparison of patients and the control groups. Student t-test was used to compare between each two variables.

RESULTS & DISCUSSION
Table 1 shows the comparison between serum levels of malondialdehyde, and trace elements in control group and patients. Table 1 reveals that the serum level of Zn has been significantly decreased in patients as compared to controls (66.50 + 0.85, p<0.001). The significance level was high in group III subjects as compared to other two groups. we did not find any significant difference in serum Mg level in all three groups (2.01 + 0.02, p>0.05). serum MDA were significantly enhanced in group II and group III subjects as compared to group I and was highest in group III (MDA : 175 + 2.69, p<0.001.

Table1 : Comparison between serum levels of malondialdehyde and trace elements in control group and patients

Parameters	Group I (N=30) (Mean + SD)	Group II (N=30) (Mean + SD)	Group III (N=30) (Mean + SD)	p-value
Trace elements				
Zn	66.50 + 0.85	58.78 + 0.64	47.95 + 0.58	P<0.001
Mg	2.01+0.02	1.97+0.02	1.96+0.02	p>0.05
Lipid peroxidation				
MDA	175.10+2.69	260.10+4.74	347.60 +4.31	P<0.001

Nutrients can affect oxidative stress by decreasing free radicals and increasing antioxidants. It is recommended that an organized study should be conducted to assess the supplementation of antioxidant nutrients and vitamins to pregnant women as prophylaxis. Hence, we conducted a comparative study of serum levels of trace elements (zinc and magnesium and malondialdehyde in 30 non-pregnant women as controls, 30 normal pregnant women and 30 women with preeclampsia.

Zinc is required for the proper functioning of anti-oxidant enzymes which protect free radicals injury. In the present study the serum zinc levels were significantly different in group I and group II, the levels being lower in group II. The serum levels of zinc were also significantly lower in group III when compared to group I. further there was significant difference in serum levels of group II and group III, the values being lower in group III. The p value being <0.001 in all the three comparisons. These findings are similar to the findings of O. Akinloye et al. [8]. Lower serum concentrations of zinc in preeclamsia compared to controls have been shown in two other relatively small retrospective studies from Turkey (mean + SD: 10.6 + 4.4 versus 12.7 +ug/L, respectively [22].

Moreover, in a retrospective study in India, reduced serum zinc concentrations in preeclamptic mothers compared to controls were reported; the authors suggest that the reduction could not only effect the antioxidant protection but could also contribute to the rise in blood pressure [23]. The lower serum zinc concentrations in mothers who develop Preeclamsia have been suggested to at least be partly due to reduced oestrogen and zinc binding-protein levels [24].

In the present study there is no significant difference in serum magnesium levels in group I, group II and group III, the p value being 0.2927. Similar were the findings of Golmohammed et al [25]. This finding is supported by Cochrane review of magnesium supplementation trials that found no evidence of benefit [26]. In the present study significant negative correlation of serum zinc with preeclamptic women suggest strong relationship between deficiency of this trace element and risk of preeclampsia, Research findings from other studies suggest that there is a relationship between nutritional status and the onset or Progress of the disease and nutitional deficiency might be involved in this disorder. It has been also cited that preeclampsia is more common in poor women. In addition, nutrients can modulate oxidative stress by increasing or decreasing free radicals or antioxidants or by providing substrate for the formation of free radicals [4].

In the present study the serum malondialdehyde levels were significantly different in group I and group II, the levels being higher in group II. The serum levels of malondialdehyde were also significantly higher in group III when compared to group I. further there was significant difference in serum levels of group II and group III, the values being higher in group III. The p value being <0.001 in all the three comparisons.

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 enzyme like superoxide dismutase, glutathione peroxidase and decrease in concentration of antioxidants.

Conclusion
The serum levels of antioxidant trace element zinc were found to be significantly decreased. There was no significant difference in serum magnesium levels in controls and cases. The indicator of lipid peroxidation, serum malondialdehyde was significantly raised levels of antioxidant ferroxidase. The findings suggest that lipid peroxidation along with plasma antioxidants and oxidants may be important factors in the pathogenesis of preeclampsia. These findings have implications for better understanding of preeclampsia and suggest that the oxidative stress has role in pathogenesis of preeclampsia.

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