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Evaluation of Serum Levels of Trace Elements, Malondialdehyde, Ceruloplasmin in the Development of Preeclampsia

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Abstract:

Preeclampsia is a pregnancy-specific condition that increases maternal and fetal mortality and morbidity. Despite its prevalence and severity, the patho-physiology of this multisystem disorder is poorly understood and its etiology has not yet been fully elucidated. This study was aimed to evaluate the role of trace elements copper, zinc, magnesium, antioxidant ceruloplasmin (Cp) and lipid peroxidation product malondialdehyde (MDA) in the pathogenesis of preeclampsia. The results showed increased levels of serum copper, Cp and MDA in normal pregnant women (group II) and in pregnant women with preeclampsia (group III) when compared to normal non pregnant women (group I), (P< 0.001). Further these levels were higher in group III when compared to group II (P< 0.001). Serum levels of zinc were significantly decreased in group II when compared to group I (P< 0.001) and were significantly lower in group III when compared to group II (P< 0.001). There was no significant difference in serum magnesium levels in controls and cases (P= 0.2927). The findings suggest that lipid peroxidation along with imbalance of antioxidants of micronutrients may be an important factor in the pathogenesis of preeclampsia.

Keywords: ***Preeclampsia, trace elements, MDA, Cp.***

Introduction:

Preeclampsia is clinically defined as hypertension and proteinuria with onset following the 20th week of pregnancy[1]. The vascular endothelium is the target for the disease process involved in preeclampsia. Maternal vascular endothelial dysfunction may be the cause of altered vascular reactivity, vasospasm and platelet aggregation[2]. Oxidative stress is a component of preeclampsia, which could provide the linkage between decreased placental perfusion and the maternal syndrome[3].

A number of micronutrients function as essential cofactors for or themselves act as antioxidants. Oxidative stress is generated during normal placental development: however, when supply of antioxidant micronutrients is limited, exaggerated oxidative stress within both the placenta and maternal circulation occurs, resulting in adverse pregnancy outcomes[4]. Deficiencies of trace elements such as zinc, copper, selenium and magnesium have been implicated in preeclampsia[5].

Copper metalloenzymes are involved in metabolic reactions, angiogenesis, oxygen transport and antioxidant protection[4]. Abnormal copper metabolism may be associated with intrauterine fetal growth restriction, preeclampsia and neurological sequelae[6]. In pregnancy, copper levels in maternal serum rise, more or less in parallel with increases in serum ceruloplasmin[7]. Zinc is required for the proper functioning of antioxidant enzymes which protect free radical injury. Deficiency of this element may withdraw the effect of antioxidant potential of cells leading to increase in blood pressure[8]. Magnesium is a unique calcium antagonist and has antiarrhythmic effect and can influence blood pressure levels by modulating vascular tone[9,10]. However, the importance of magnesium-induced vasodilation in the treatment and prevention of eclampsia is not completely understood[10].

Ceruloplasmin is a ferroxidase and its ferroxidatic activity converts toxic ferrous iron to less toxic ferric iron, thereby reducing oxidative damage to lipids, proteins and DNA[11]. Placental hypoxia associated with preeclampsia, increases placental ceruloplasmin expression. The role of this high oxidizibility in the pathogenesis of preeclampsia has yet to be evaluated[12]. MDA is the end product of lipid peroxidation and reflects the oxidative status of the biological system[13]. Elevated levels of oxidative lipid derivatives and reduced antioxidants in the circulation of preeclamptics may contribute to endothelial damage[14].

The conflicting result of studies evaluating the effects of trace elements and antioxidants on clinical outcomes of preeclampsia indicates that the regulatory behavior of trace elements and antioxidants needs to be validated. Therefore the present study was intended to identify the level of trace elements (copper, zinc and magnesium) in normal non pregnant, normal pregnant and women with preeclampsia

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and to know the changes in oxidative stress by measuring the antioxidant ceruloplasmin and the product of lipid peroxidation, MDA.

Materials and Methods:

The present study was conducted at Department of Biochemistry in association with Obstetrics and Gynecology Department of Princes 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: 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 by 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, hypertension) or taking medications that could potentially affect levels of trace elements were also excluded.

Sample collection and serum separation

Blood samples (5ml) were collected from the cubital vein using sterile needle and syringe into plain tubes. The samples were allowed to clot undisturbed and serum was separated by centrifugation for 10 minutes at 3000 rpm into plain tubes and stored at -20°C until time of analysis.

Biochemical analysis

Serum copper and zinc were analyzed by colorimetric methods[15,16] on Semiautoanalyzer, Microlab 300. Serum magnesium (xylidyl blue method) and serum ceruloplasmin (Immunoturbidimetric assay) were analyzed on Cobas C311 auto analyzer[17, 18]. MDA was measured by Thiobarbituric acid reactive substance Assay (TBARS)[61].

Statistical analysis:

All the results were expressed as mean±standard deviation (SD). Statistical analysis was carried out using Graph Pad Prism (version V) and Microsoft 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

Serum levels of trace elements

The serum level of Zn was significantly decreased in patients as compared to controls (66.50±0.85, $p<0.001$). The decrease being more in group III subjects as compared to other two groups. Whereas serum Cu levels were significantly enhanced in group II and group III subjects as compared to group I. The increase in Cu level was highest in group III subjects as compared to other two groups (122±1.15, $p<0.001$). We did not find any significant difference in serum Mg level in all three groups (2.01±0.02, $p>0.05$).

MDA and Ceruloplasmin

Serum MDA and Cp 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, $p<0.001$ and Cp: 60.16±0.88, $p<0.001$).

Table: 1. Comparison between serum levels of Malondialdehyde, Ceruloplasmin 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
Cu	122.83 ± 1.15	268.00 ± 5.28	302.63 ± 4.17	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
Antioxidant				
Cp	6.16 ± 0.88	114.95 ± 1.56	139.70 ± 2.51	p < 0.001

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Figure: 1. Serum levels of Trace Elements

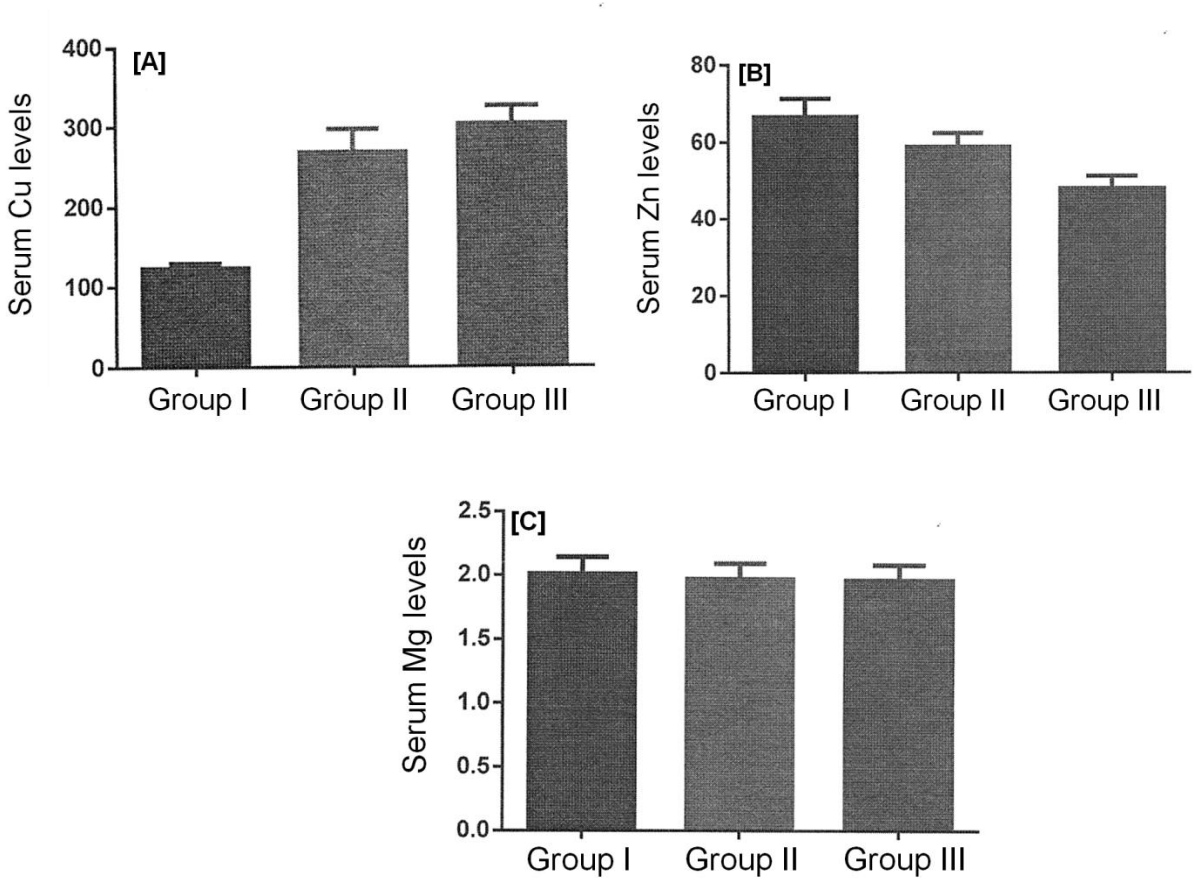
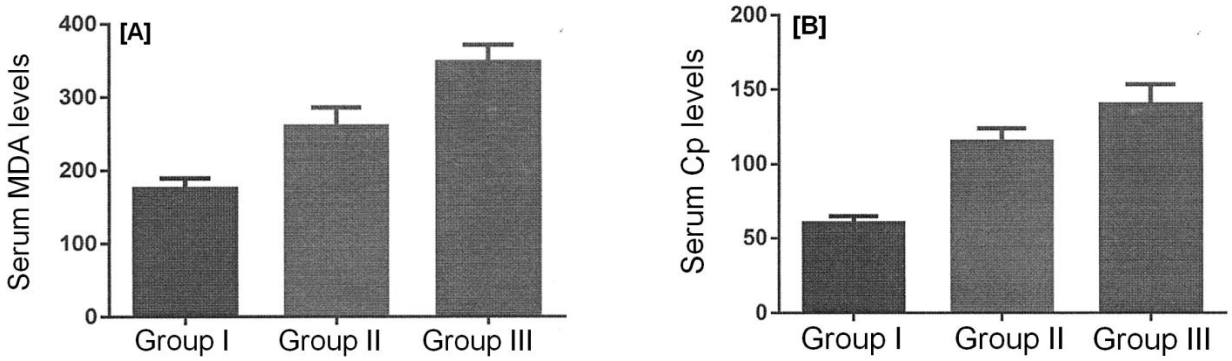


Figure 2: Serum levels of Malondialdehyde and Ceruloplasmin



Discussion:

The clinical manifestations of hypertension and proteinuria that define preeclampsia probably represent the late stage of a disease that begins very early in pregnancy and it is likely that many different initial insults converge on a common pathophysiology[1]. However, the placenta is armed with antioxidant defenses, including copper/zinc and manganese superoxide dismutases which protect the placenta from any undue harm[20].

Copper can act as both an antioxidant and a pro-oxidant[21]. In the present study a significant difference was observed in serum copper levels in group I (non-pregnant women) and group II (normal pregnant women), the levels being higher in group II (P, 0.001). Further the serum copper levels of group III were significantly increased the increase being more than that of group II (P < 0.001). These findings are similar to the retrospective studies from Turkey[22] and study of Thompson and Watson[6]. In the study by Jelka Vukelic, Aleksandra Kapamadzija, *et al.* healthy pregnant women with normal course of pregnancy showed a constant trend of the increase of the mean serum copper values compared to the mean serum copper values in healthy non-pregnant wome[7]. Copper delivery to the developing foetus by the specific transporters in the placenta is regulated by the mother's estrogen and insulin levels[23]. Amongst various factors affecting copper level, elevated levels of oestrogen during pregnancy increase the synthesis of ceruloplasmin by

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making copper available through mobilization from maternal tissues especially liver [24].

Zinc is required for the proper functioning of antioxidant enzymes which protect free radicals injury. Deficiency of this element may withdraw the effect of antioxidant potential of cells leading to increase in blood pressure[25]. 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* [5]. Lower serum concentrations of zinc in preeclampsia compared to controls have been shown in two other relatively small retrospective studies from Turkey (mean ± SD: 10.6 ± 4.4 versus 12.7 ± 4.1µg/L, respectively) [26]. Moreover, in a recent 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 [27].

The lower serum zinc concentrations in mothers who develop preeclampsia have been suggested to at least be partly due to reduced oestrogen and zinc binding-protein levels[28]. 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 suggests that there is a relationship between nutritional status and the onset or progress of the disease and nutritional 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[29].

The success of magnesium therapy as a treatment for eclamptic seizures and the known effect of magnesium on vascular responses in vitro suggested that magnesium might be deficient in women with preeclampsia[29]. In a study by S. Gulmohammad et al the mean serum levels of magnesium in normotensive pregnant women and preeclamptic women were not significantly different[30]. In the present study there was no significant difference in serum magnesium levels in group I, group II and group III, the P value being 0.2927. Earlier studies by Kumru *et al.* also found similar results[26]. The Cochrane review including two trials showed no apparent effect of magnesium supplementation on the prevention of eclampsia. The authors of the review concluded that dietary magnesium supplementation of pregnant women cannot be recommended for routine clinical practice because of the poor methodological quality of the current evidence[31]. This is, of course, unrelated to the effectiveness of parenteral magnesium sulfate for treatment of preeclampsia and eclampsia demonstrated in two large randomized control trials[32,33]. The data available suggest if magnesium deficiency does occur in preeclampsia, it is a result rather than a cause of the disorder and that supplementation is unlikely to be beneficial[29].

Ceruloplasmin is a ferroxidase[34] and its ferroxidatic activity is an important function of this enzyme because even trace amounts of iron can produce hydroxyl radicals through the Fenton reaction which can destroy cellular architecture. Ferroxidatic activity of ceruloplasmin is known to convert toxic ferrous iron to less toxic ferric iron, which reduces oxidative damage to lipids, proteins and DNA [35].

In the present study there was significant difference in serum ceruloplasmin levels in group I and group II (P value < 0.001), the levels being higher in group II. The serum levels of ceruloplasmin were also significantly higher in group III when compared to group I (P value < 0.001). Further there was significant difference in serum levels of group II and group III (P value < 0.001), levels being higher in group III. Compatible with present study results, Fattah et al. (1976) showed that ceruloplasmin levels were significantly elevated in the maternal blood of preeclamptic patients as compared with normal pregnant women[36]. Aksoy *et al.* (2003) also reported increased ceruloplasmin levels in preeclamptic women compared to those with the healthy pregnant group. Microarray studies detected an up-regulation of mRNA for ceruloplasmin, in preeclamptic placentas compared to preterm and term controls. Quantitative realtime PCR confirmed these results. To date, it is not known whether placental ceruloplasmin is secreted, thus its potential contribution to the preeclampsia-associated increase of this protein in maternal serum is unknown. The question then arises as to which factors may lead to increased placental ceruloplasmin expression in preeclampsia. One possibility is that placental hypoxia which accompanies preeclampsia increases placental ceruloplasmin expression as has been noted in studies using macrophages and monocytes. Concerning the potential role of elevated placental ceruloplasmin in preeclampsia, it is known that this syndrome is characterized by increased placental expression of ROS, lipid peroxidation, and damage to villous architecture. Increased levels of placental ceruloplasmin in preeclampsia would result in enhanced ferroxidatic activity, thereby converting excess ferrous iron to the less toxic ferric form. This suggests that syncytial ceruloplasmin, induced by hypoxic conditions in preeclampsia, plays a key role in a cellular program which

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mitigates the damaging effects of subsequent reperfusion injury in placenta[11]. Ceruloplasmin is an acute phase reactant and its concentration in serum is upregulated during infection, inflammation and tissue trauma, mediated by inflammatory cytokines[29]. Its expression has also been shown to increase under hypoxic conditions[37]. The high ceruloplasmin levels in late stages of pregnancy suggest an enhanced catecholamine breakdown during the stress alarm reaction[38]. It has been suggested that increased placental expression of the antioxidant enzymes may serve as an adaptive or protective mechanism to limit oxidative damage in preeclampsia. Increased levels of placental ceruloplasmin in preeclampsia may result in enhanced ferroxidative activity in this tissue, thereby oxidizing excess ferrous iron to the less toxic ferric form[12].

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 MDA was significantly elevated in preeclamptics compared to healthy pregnant women (p < 0.001) in the study by Usha Adiga *et al.*(2007)[39]. The values obtained are in close agreement with those reported by Aydin *et al* [14]. Meera *et al* (2010) also observed similar raised serum malondialdehyde values in preeclamptics when compared to controls. Increased oxygen demand to meet the bodily functions in pregnancy is also a contributory factor for the oxidative stress that results in the formation of free radicals. Thus, lipid alterations observed may promote oxidative stress, leading to endothelial dysfunction in preeclampsia. There is good evidence for significant increase in the levels of plasma and erythrocyticmalondialdehyde, a marker of lipid peroxidation in normotensive pregnant women, which were further increased in women with pregnancy induced hypertension. Evidence of increased oxidative lipid derivatives in the decidual placental tissues was observed in women with established preeclampsia. Elevated levels of oxidative lipid derivatives, conjugated dienes and reduced antioxidative capacity in the maternal circulation have also been reported[40].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. Reactive oxygen species can cause enhanced lipid peroxidation[13]. Lipid peroxides are directly involved in mediating maternal endothelial dysfunction by increasing the production of thromboxane A2 and the expression of cell adhesion molecules in the uteroplacental vasculature as well as in the maternal peripheral vasculature[24].

Conclusion

In this comparative study of serum levels of trace elements (copper, zinc, magnesium), ceruloplasmin and malondialdehyde, the serum levels of antioxidant trace element zinc was found to be significantly decreased. .Serum copper levels were significantly raised which may be due to the increased synthesis of ceruloplasmin. There was no significant difference in serum magnesium levels in controls and cases. Further widespread studies may be undertaken to establish the role of magnesium, if present in preeclampsia. The indicator of lipid peroxidation, serum malondialdehyde was significantly raised along with significant raised levels of antioxidant ferroxidase, ceruloplasmin. The findings suggest that lipid peroxidation may be an important factor in the pathogenesis of preeclampsia and that plasma antioxidants and oxidants are altered in preeclampsia.

These findings have implications for better understanding of preeclampsia. And suggest that the oxidative stress has role in pathogenesis of preeclamsia. In pregnancy-specific conditions like preeclampsia, the knowledge of etiology is limited and the best strategy would be to continue to test promising interventions in populations with and without nutritional deficiency in a major coordinated research effort based on a systematic review of the literature that is focused and can address these problems.

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