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Serum Level and Antioxidant Activity of Ceruloplasmin in Preeclampsia

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Abstract: The antioxidants activities are decreased in the serum of women with preeclampsia. This study was aimed to determine the serum level and antioxidant activity of ceruloplasmin in preeclamptic women with gestational age over than 28 weeks. In a cross-sectional and descriptive-analytic study performed on 60 patients with preeclampsia (30 with mild and 30 with severe preeclampsia) and 30 women with normal pregnancy (control) in Tabriz al-Zahra Hospital, serum level and antioxidant activity of ceruloplasmin was evaluated. The mean gestational age was 32.94±2.79 week in mild preeclampsia group, 32.17±3.00 week in severe preeclampsia group and 32.46±4.04 week in control group (p = 0.821). The mean serum level of ceruloplasmin was 0.62±0.16 g L^{-1} in mild preeclampsia group, 0.61±0.23 g L^{-1} in severe preeclampsia group and 0.47±0.16 g L^{-1} in control group. The serum ceruloplasmin in control group was significantly lower (p = 0.006). The mean antioxidant activity of ceruloplasmin was 562.54±139.79 in mild preeclampsia group, 556.21±190.94 in severe preeclampsia group and 427.62±162.14 in control group. The antioxidant activity was measured as production of mg dL^{-1} of a colored product. The antioxidant activity of ceruloplasmin in control group patients was significantly lower (p = 0.002). Significant linear positive correlation was found between serum level of ceruloplasmin and antioxidant activity of ceruloplasmin (p<0.001 and r = 0.910). Serum level of ceruloplasmin is significantly lower in normal pregnancy than mild and severe preeclampsia. Antioxidant activity of ceruloplasmin is significantly lower in normal pregnancy than mild and severe preeclampsia.

Key words: Preeclampsia, ceruloplasmin, antioxidant activity, pregnancy, oxidative stress

INTRODUCTION

Preeclampsia (PE) accounts for about one-quarter of maternal mortality and the second cause of pregnancy-associated maternal deaths in worldwide. So, the identification of an effective strategy to prevent PE is a priority and a challenge for research in obstetrics (Xu *et al*., 2009). Unfortunately, the precise etiology of PE remains unknown (Xu *et al*., 2009; Guller *et al*., 2008; Acikgoz *et al*., 2006). There is consensus that ischemia/reperfusion injury associated with PE promotes both placental damage and the release of factors leading to maternal endothelium dysfunction, a hallmark of this potentially life-threatening syndrome (Guller *et al*., 2008). Studies have demonstrated the presence of oxidative stress and inflammation in PE. Some oxidative and inflammatory mediators were altered in preeclampsia and some correlated to blood pressure (Bernardi *et al*., 2008).

Increasing evidences suggests that oxidative stress may play a key role in the etiology of PE (Ruder *et al*., 2009; Wruch *et al*., 2009; Talaulikar and Manyonda, 2009). Recent studies carried out on preeclampsia have focused on the increase in free radicals in the feto-placental unit with poor perfusion (Acikgoz *et al*., 2006; Atamer *et al*., 2005). Plasma Antioxidant Potential (AOP) of the severe and mild preeclampsia groups were found to be reduced and plasma antioxidants and oxidants are altered in preeclampsia (Aksoy *et al*., 2003). Several reports suggest PE to be associated with oxidative stress. High levels of serum hydroperoxides and increased susceptibility of serum lipids to peroxidation indicate PE to be associated with high oxidative stress. The role of this high oxidizibility in the pathogenesis of PE has yet to be evaluated (Atamer *et al*., 2005; Fainaru *et al*., 2003).

Several studies have used gene array technology to identify alterations in placental gene expression (Soleymanlou *et al*., 2005; Hansson *et al*., 2006; Reimer *et al*., 2002; Zhou *et al*., 2006) and have examined the role of oxidative stress (Ruder *et al*., 2009) associated with PE (Guller *et al*., 2008; Engin-Ustün *et al*., 2005; Orhan *et al*., 2001). It is interesting to speculate as to the potential role of elevated placental ceruloplasmin in PE. The disorder is characterized by increased placental expression of ROS, lipid peroxidation and damage to villous architecture (Walsh *et al*., 2000; Myatt and Cui, 2004; Myatt *et al*., 1996). Studies showed that placental

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and serum levels of ceruloplasmin are increased in pregnancies with severe PE (Guller *et al*., 2008; Engin-Ustün *et al*., 2005; Orhan *et al*., 2001). This conclusion is based on several experimental evidences indicating that placental ceruloplasmin, a protein with antioxidant properties (Guller *et al*., 2008; Hellman and Gitlin, 2002; Patel *et al*., 2002) expression is clearly upregulated in the PE group vs. patients matched for gestational age (Guller *et al*., 2008). Possibly placental hypoxia associated with PE (Redman and Sargent, 2005; Kaufmann *et al*., 2003) increases placental ceruloplasmin expression as has been noted for macrophages and monocytes (Sarkar *et al*., 2003).

It has been suggested that increased placental expression of the antioxidant enzymes may serve as an adaptive/protective mechanism to limit oxidative damage in PE (Wang and Walsh, 1996; Knapen *et al*., 1999). Increased levels of placental ceruloplasmin in PE may result in enhanced ferroxidatic activity in this tissue, thereby oxidizing excess ferrous iron to the less toxic ferric form (Guller *et al*., 2008). There is consensus that PE is strongly associated with failed conversion of maternal endometrial spiral arteries in the placental bed (Redman and Sargent, 2005; Kaufmann *et al*., 2003). Ischemic placental tissue may be a primary source of potentially toxic agents in preeclampsia and the released iron species may contribute to the etiology and would exacerbate lipid peroxidation and endothelial cell injury, which may be abated by antioxidant supplementation (Serdar *et al*., 2006). Placental ceruloplasmin, induced by hypoxia associated with severe PE, may be important in an endogenous cellular program to lower the damaging effects of subsequent reperfusion injury at this site (Guller *et al*., 2008).

This study was aimed to determine the serum level and antioxidant activity of ceruloplasmin in preeclamptic women with gestational age over than 28 weeks.

MATERIALS AND METHODS

This is a cross-sectional and descriptive-analytical study performed on pregnant women with mild or severe preeclampsia and women with normal pregnancy presenting to Tabriz Al-Zahra Hospital from 2006 to 2007.

The samples were selected from consecutive preeclamptic women with gestational age of more than 28 weeks. The inclusion criteria were: (1) pregnant women with gestational age of >28 weeks and (2) proteinuria with hypertension. The exclusion criteria were: (1) systemic disease (e.g. UTI), (2) HELLP syndrome, (3) twins pregnancy, (4) obstetrics or drug side effects, (5) molar pregnancy, (6) use of anti hypertensive drugs and (7) substance or opiate abuse (Safaei, 2008).

According the degree of hypertension and proteinuria, the patients were classified as mild and severe preeclampsia group. So, we enrolled 30 patients with mild preeclampsia (Group 1) and 30 patients with severe preeclampsia (Group 2) which were compared with 30 healthy pregnant women with gestational age of >28 weeks and without preeclampsia (Group 3 or Control).

The patients' variables were recorded including age, gravidity, parity, maternal serum ceruloplasmin, antioxidant activity of maternal plasma ceruloplasmin, maternal symptoms and signs and blood pressure. After obtaining a full history and performing physical examination and filling the questionnaire, 5cc blood was sampled from patients. The samples then were sent to Laboratory of Al-Zahra Hospital for centrifuge and isolation of serum and plasma.

Then the serum was sent in to Tabriz Danesh Laboratory for measurement of serum ceruloplasmin and the frozen plasma was sent to Department of Drug Applied Research Center in Tabriz University of Medical Science for detection of antioxidant activity of maternal plasma ceruloplasmin.

The serum ceruloplasmin was measured by Minineph Human Ceruloplasmin Kit made by The binding site, Ltd., Birmingham, UK and was expressed as g L^{-1}.

The antioxidant activity of maternal plasma ceruloplasmin was detected by oxidant PPD (para-phenylene diamine) and Na-acetate and production of colored products as mg dL^{-1}. In this method, the blood plasma was isolated by centrifuge for 15 min in 25°C and then was frozen in -20°C. One milliliter of PPD is solved in 1 cc of acetate buffer and 1 cc of the prepared solution is added to 0.1 cc of sample; the mixed solution is incubated in 37°C for 15 min. The reaction is stopped with 5cc of azid 0.02%.

One standard sample is produced by incubation of 1cc of PPD for 15 min in 37°C and adding 0.1 cc of plasma into PPD and then, rapid stopping of reaction by adding 5 cc of azid 0.02%. The colored products are measured by a spectrophotometer in absorption spectrum of 540 nm. The enzymatic activity is detected by difference of absorption between test and standard samples.

The collected data was analyzed by SPSS-13 statistical software using t-test for comparison of average values, Chi-square and Mann-Whitney-U test for evaluation of qualitative variables and one-sided variance analysis for analysis of hypotheses. The results were expressed as percent and mean with standard deviation. The p-values of less than 0.05 was considered as statistically significant. Data were analysed statistically using one-way analysis of variance and Pearson correlation test. Logistic regression procedures were used to calculate odds ratios (Serdar *et al*., 2006; Safaei, 2008).

Table 1: The comparison of parameters in three studied groups

Variable	Mild preeclampsia	Severe preeclampsia	Control group	p-value
Age (year)	28.48±5.12	30.71±6.34	27.80±4.81	0.082
Gravidity	2.12±1.59	2.48±1.46	1.83±0.87	0.161
Parity	0.98±0.74	1.45±1.37	0.76±0.72	0.034
Gestational age (w)	32.94±2.79	32.17±3.00	32.46±4.04	0.821
24 h urine protein (g)	782.86±319.51	1256.78±835.05	751.46±216.42	0.192
Serum ceruloplasmin (g L^{-1})	0.62±0.16	0.61±0.23	0.47±0.16	0.006
Antioxidant activity (mg dL^{-1})	562.54±139.79	556.21±190.94	427.62±162.14	0.002

RESULTS

The studied patients had the mean age of 29.06±5.58 year, the mean gravidity of 2.16±1.37, the mean parity of 1.14±0.97 and the mean pregnancy age of 32.52±3.38 week in admission.

The main manifestations in admission were headache (5 cases in mild and 9 cases in severe preeclampsia), blurred vision (3 cases in mild and 2 cases in severe preeclampsia) and epigastric pain (2 cases in mild and 1 case in severe preeclampsia).

The average 24 h protein was 5923.37±1278.33 g. The average serum ceruloplasmin was 0.57±0.20 g L^{-1}, with average antioxidant activity of 1752.45±516.88.

Blood pressure of = 140/90 mmHg was occurred in 34 cases in mild and 8 cases in severe preeclampsia groups. Blood pressure of = 160/110 mmHg was occurred in one case in severe preeclampsia group.

Table 1 shows the comparison of parameters in three studied groups.

One way ANOVA test showed that the difference of age, gravidity, gestational age and 24h urine protein was not significantly different in studied groups (Table 1), but the difference of parity, serum ceruloplasmin and antioxidant activity was significantly different between three groups (Table 1).

There was a significant direct relation between age and gravidity ($p<0.001$, $r = 0.521$), age and parity ($p<0.001$, $r = 0.521$), gravidity and parity ($p<0.001$, $r = 0.852$), serum ceruloplasmin and its antioxidant activity ($p<0.001$, $r = 0.910$).

However, we did not found significant direct relation between patient age and gestational age ($p = 0.072$, $r = -0.181$), age and serum ceruloplasmin ($p = 0.297$, $r = 0.110$), age and Antioxidant activity of ceruloplasmin ($p = 0.567$, $r = 0.061$), age and urine 24 h protein ($p = 0.567$, $r = 0.061$), GA and gravidity ($p = 0.477$, $r = -0.072$), gravidity and serum ceruloplasmin ($p = 0.203$, $r = 0.134$), gravidity and antioxidant activity ($p = 0.406$, $r = 0.088$), gravidity and 24 h urine protein ($p = 0.493$, $r = 0.087$), parity and GA ($p = 0.330$, $r = -0.098$), parity and serum ceruloplasmin ($p = 0.348$, $r = 0.099$), parity and antioxidant activity ($p = 0.591$, $r = 0.057$), parity and 24 h urine protein ($p = 0.399$, $r = -0.106$), GA and serum ceruloplasmin ($p = 0.76$, $r = 0.027$), GA and antioxidant activity ($p = 0.958$, $r = -0.006$), GA and 24 h urine protein ($p = 0.856$, $r = -0.023$), serum ceruloplasmin and 24 h urine protein ($p = 0.932$, $r = -0.011$), antioxidant activity and 24 h urine protein ($p = 0.770$, $r = 0.039$).

DISCUSSION

Where oxidative stress plays an important role in the pathogenesis of many chronic diseases (Suresh *et al*., 2008), antioxidants have received increased attention by nutritionists and medical researchers for their potential effects in the prevention of chronic and degenerative diseases (Al-Humaid *et al*., 2010). Ceruloplasmin (Cp) is a copper-containing iron transport protein with antioxidant ferroxidase properties (Guller *et al*., 2008; Hellman and Gitlin, 2002; Patel *et al*., 2002). It was first described as a member of a copper-containing oxidase family of enzymes (Hellman and Gitlin, 2002). However, Cp is not involved in the transport or metabolism of copper (Hellman and Gitlin, 2002; Redman and Sargent, 2005). Ceruloplasmin is also a ferroxidase (Osaki *et al*., 1966) 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 (Hellman and Gitlin, 2002; Patel *et al*., 2002). 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 (Hellman and Gitlin, 2002; Patel *et al*., 2002). The Cp is an acute phase reactant and its concentration in serum is upregulated during infection, inflammation and tissue trauma, mediated by inflammatory cytokines (Guller *et al*., 2008). Its expression has also been shown to increase under hypoxic conditions (Sarkar *et al*., 2003). The high ceruloplasmin levels in late gestoses suggest an enhanced catecholamine breakdown during the stress alarm reaction (Fuchs *et al*., 1991).

In agreement with our findings previous studies showed that Cp levels are significantly elevated not only in preeclampsia but also in patients with essential hypertension (Griffin, 1983). The antioxidant activity of Cp decreases with increasing transferrin saturation by iron. Increased transferrin saturation and decreased

unsaturated iron-binding capacity in preeclampsia may occur consequent to oxidative stress and then further promote oxidative stress by decreasing serum antioxidant buffering against redox-active iron (Hubel *et al*., 1996). Compatible with our study results, Fattah *et al.* (1976) showed that Cp levels were significantly elevated in the maternal blood of pre-eclamptic patients as compared with normal pregnant women.

Vitoratos *et al.* (1999) studied 30 primigravidas between 32 and 36 weeks of gestation to investigate the role of serum ceruloplasmin in PE. Compatible with our study results, the preeclamptics presented significantly higher serum Cp levels compared to those with normal pregnancies, while the mean ferroxidase activity levels of ceruloplasmin did not differ significantly between the two groups. Thus, it seems that the plasma of preeclamptic women lacks the protective anti-oxidative action of these substances (Vitoratos *et al*., 1999). In present study, antioxidant activity of ceruloplasmin was significantly lower in normal pregnancy than mild and severe preeclampsia.

Aksoy *et al.* (2003) studied 21 patients with mild preeclampsia, 15 patients with severe preeclampsia and 19 normotensive pregnant women. Whereas the plasma antioxidant potential (AOP) of the severe and mild preeclampsia groups were found to be reduced, the Cp levels were increased compared with those of the healthy pregnant group. The findings suggested that lipid peroxidation may be an important factor in the pathogenesis of preeclampsia and that plasma antioxidants and oxidants are altered in preeclampsia (Aksoy *et al*., 2003). In their study, no differences were observed between the groups with severe and mild preeclampsia. Their study is compatible with ours in which we did not found any difference between severe and mild preeclampsia about ceruloplasmin level and its antioxidant activity.

Ischemic placental tissue may be a primary source of potentially toxic iron in preeclampsia and the released iron species may contribute to the etiology and would exacerbate lipid peroxidation and endothelial cell injury, which may be abated by antioxidant supplementation (Serdar *et al*., 2006). Serdar *et al.* (2006) studied 30 healthy, 30 mild preeclamptic and 30 severe preeclamptic pregnant women and found Significant correlations between serum iron and lipid peroxides in serum in severe preeclamptic pregnancy (Serdar *et al*., 2006). In another study, Cp and its oxidase activity were determined in serum from 52 women in the last trimester of normal pregnancy and in 50 control women of similar age. Similar tour study, serum levels of Cp and its oxidase activity were significantly higher in pregnant women than control group (Louro *et al*., 2001). In pregnant women, the specific oxidase activity for Cp (364.4±3.3 vs. 407.5±3.8 U g^{-1}) was significantly lower than in the control group. They concluded that pregnancy accelerates the rate of ceruloplasmin protein synthesis and release (Louro *et al*., 2001). In our series, antioxidant activity of ceruloplasmin was significantly lower in normal pregnancy than mild and severe preeclampsia.

Mora *et al.* (1976) measured serum ceruloplasmin by oxidase and immunochemical methods in three groups of people, normal, pregnant women and their newborns. Ceruloplasmin showed a an increase in pregnant women as compared with the normal controls (Mora *et al*., 1976). Increased lipid peroxidation is well correlated with the increase in systolic and diastolic BP measurements (Yanik *et al*., 1999). The specific oxidase activity of ceruloplasmin decreased in pregnant women with gestosis, feto-placental insufficiency and postmature. The ceruloplasmin concentration decreased in the postmature pregnancy (Krainova *et al*., 2005).

In Vitoratos *et al.* (1999) study, the preeclamptic women presented significantly higher serum ceruloplasmin levels than normal pregnants, while the mean ferroxidase activity of Cp did not differ significantly between two groups. The mean serum iron level was greater whereas the total iron binding capacity was lower in preeclamptics than normal pregnants. Thus, it seems that the plasma of preeclamptic women lacks the protective anti-oxidative action of these substances (Vitoratos *et al*., 1999).

Guller *et al.* (2008) characterized placental factors involved in the pathophysiology of PE. They suggested that syncytial ceruloplasmin and its associated ferroxidase activity, induced by the hypoxia accompanying severe PE, is important in an endogenous cellular program to reduce the damaging effects of subsequent reperfusion injury at this site (Guller *et al*., 2008). Increased transferrin saturation and decreased unsaturated iron-binding capacity in preeclampsia may occur consequent to oxidative stress and then further promote oxidative stress by decreasing serum antioxidant buffering against redox-active iron (Hubel *et al*., 1996).

Microarray studies detected an upregulation of mRNA for ceruloplasmin in PE compared to both Preterm Control (PC) and Term Control (TC) groups' placentas, respectively (Guller *et al*., 2008). The PCR confirmed these results by demonstrating significant increases in ceruloplasmin mRNA in PE vs. PC and TC placentas. PCR identified the presence of mRNA for ceruloplasmin in primary cultures of syncytiotrophoblasts, but not villous-derived fibroblasts, suggesting that syncytium is the site of ceruloplasmin synthesis in placenta (Guller *et al*., 2008). Immunohistochemistry localized

ceruloplasmin to the intervillous space in PE and PC placentas, whereas stronger syncytial staining was noted in PE. Western blotting confirmed a significant increase in ceruloplasmin levels in placental tissue in PE compared to PC groups (Guller *et al*., 2008).

Elevation of lipid peroxides together with impaired antioxidant defense mechanisms may be related at least partly to the pathogenesis of preeclampsia. Additionally, lipid peroxides and blood oxidative imbalance could be part -of the cytotoxic mechanisms leading to endothelial cell injury (Atamer *et al*., 2005). Lipid peroxides in serum and placental tissue and ceruloplasmin levels in serum are significantly increased. There are significant correlations between serum malondialdehyde and ceruloplasmin in women with severe preeclampsia and changes in serum and placental lipid peroxides and serum iron concentrations are significantly associated with preeclampsia (Serdar *et al*., 2006). The Plasma antioxidant potential (AOP) of the severe and mild preeclampsia patients were found to be reduced, but Cp levels were increased compared with those of the healthy pregnant group. Lipid peroxidation may be an important factor in the pathogenesis of preeclampsia and plasma antioxidants and oxidants are altered in preeclampsia (Aksoy *et al*., 2003). Ischemic placental tissue may be a primary source of potentially toxic iron in preeclampsia and the released iron species may contribute to the etiology and would exacerbate lipid peroxidation and endothelial cell injury, which may be abated by antioxidant supplementation (Serdar *et al*., 2006).

Syncytiotrophoblast (SCT) release of microparticles has also been suggested to play a role in regulating maternal endothelial and immune cell function. It is of note that syncytial release of the anti-angiogenic factors increases in preeclampsia, a major cause of maternal mortality and morbidity. In preeclampsia, hypoxia and reperfusion injury in the placenta is associated with activation of the maternal endothelium (Guller, 2009).

Li studied immunological parameters in 180 women including 60 normal without pregnancy, 60 normal gravidaes and 60 gravidaes with pregnancy induced hypertension. Cp in gravidaes were markedly higher than that in normal women. Cp in PIH was markedly higher than that in gravidaes. The results may be as reference materials of pregnancy induced hypertension in diagnose and treatment (Li, 1993). One study measured maternal plasma advanced oxidative protein products (AOPP) levels at 19-25 weeks of gestation. AOPP levels were not significantly different between normotensive and preeclamptic women. Second trimester plasma concentrations of plasma AOPP are not altered in women with preeclampsia later in pregnancy (Dane *et al*., 2009). Initially the oxidative stress due to pregnancy-induced hypertension is critically combated by the intricate defensive mechanism of natural antioxidant system of the body. It appears that this imbalance between oxidant and antioxidant is the effect of disease and not the causative factor (Kaur *et al*., 2008).

CONCLUSION

Serum level of ceruloplasmin is significantly lower in normal pregnancy than mild and severe preeclampsia. Antioxidant activity of ceruloplasmin is significantly lower in normal pregnancy than mild and severe preeclampsia.

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