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Maternal Serum Levels of Zinc, Copper, and Thiols in Preeclampsia Patients: a Case-Control Study

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Abstract

Preeclampsia is one of the leading causes of maternal mortality-morbidity, and environmental factors act as the main driving force for the development of disease in genetically lean women. Trace element levels (zinc, copper) and thiol state (total, native thiol) may affect involved risk factors and play a role in the pathogenesis. The objective of our study is to assess trace element and thiol levels in patient and control groups. A total number of 88 pregnant women (in their third trimester) included 43 preeclampsia patients and 45 normotensive pregnant women as controls. The main findings of this study were the significantly elevated copper levels and decreased thiol levels (native and total thiols) in the patient group compared to controls ($p < 0.05$). Disulfide levels were not statistically different between the groups ($p > 0.05$). In patients, the predictive cutoff value of copper was 224 μg/dL and was 1.19 for the copper/native thiol ratio. Zinc levels were not statistically different between the two groups. Correlation analysis revealed no relationship between zinc-copper and zinc-total thiol levels in patients, while a positive correlation was evident in controls (zinc-copper, $p < 0.05$, $r = 0.425$, and zinc-total thiol levels, $p < 0.05$, $r = 0.642$). Patients had marginally high ALT and AST values in the normal range, and a significant difference was found between the two groups ($p < 0.05$). According to these results, elevated copper levels and decreased thiol levels may have a value for early prediction. The mechanisms that may be responsible for the altered element and thiol status have been discussed here in the context of oxidative stress.

Keywords Preeclampsia · Copper · Zinc · Thiol · Oxidative stress

Introduction

Preeclampsia (PE), one of the major causes of preterm delivery and maternal morbidity/ mortality, is seen as a complication in 10% of all pregnancies. It may both present as new onset hypertension with proteinuria or new onset hypertension with end-organ dysfunction after the 20 weeks of gestation. Pathogenesis depends on the placental and maternal vascular dysfunction, and several risk factors are identified for the development of the disease. Systematical reviews showed several contributing risk factors that are associated with the disease, and clinical factors that carry the highest relative risk (RR) are found such as history of preeclampsia, multiple pregnancy, pregestational diabetes, autoimmune diseases, and chronic kidney disease [1, 2]. Increased prevalence of preeclampsia among poor women has led researchers to investigate the role of nutritional condition, but contradictory results did not provide a specific target [3]. There also is increasing

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knowledge that oxidative stress plays an important role in the pathogenesis of preeclampsia. Herewith, the micronutrient issue became prominent since nutrients can alter oxidative status by numerous mechanisms [4]. The present study investigates zinc (Zn^{2+}) and copper (Cu^{2+}), within the context of micronutrient concept and aims to contribute to literature which has conflicted results of trace elements in preeclampsia.

Trace elements constitute a small fragment of human body; however, they play a crucial role in maintaining normal metabolic function. Deficient or excess amount of these elements cause improper function of the relevant enzymes and so to various clinical manifestations. Zinc, the second most abundant trace element in body, functions as a part of multiple metalloenzymes [5]. The enzymes containing zinc in their active site are alcohol dehydrogenase, alkaline phosphatase, lactate dehydrogenase, carboxypeptidase, carbonic anhydrase, superoxide dismutase (SOD), DNA, and RNA polymerase. It is essential for prenatal-postnatal development, and both deficiency or excessive state of Zn^{2+} is related to various clinical outcomes [6]. Zn^{2+} is carried in blood by albumin (60%) and transferrin (10%) with the rest found in free form. Zn^{2+} is crucial for maintaining normal reproductive health and decreased amounts cause to serious maternal-fetal outcomes as postpartum bleeding, fetus growth restriction (FGR), fetal malformations, preterm delivery, and as discussed in this study detailed, preeclampsia [7].

Copper is another essential transition metal functioning as cofactor for multiple cuproenzymes: ceruloplasmin (Cp), lysyl oxidase, copper-zinc superoxide dismutase (Cu-Zn SOD), dopamine-β-hydroxylase, cytochrome c oxidase, and tyrosinase [3]. Around 95% of Cu^{2+} in plasma is bound to ceruloplasmin, and the free form is carried by albumin throughout the body. Clinical states associated with Cu^{2+} deficiency are diabetes, cardiovascular disease, altered cholesterol metabolism, increased frequency of infections, and Menkes disease [8]. In utero deficiency may lead to early embryonic death, structural abnormalities (permanent neurologic, cardiovascular dysfunction, and impaired bone formation), as well as immune dysfunction [9]. The effect of marginal excess of Cu^{2+} is not fully understood, but higher levels cause acute or chronic toxicity. Crucially, high levels of Cu^{2+} increases oxidative response in cells by Fenton-type redox reactions, and this fact may explain the pathogenesis of oxidative damage-related diseases that found to be associated with Cu^{2+} toxicity [10]. Studies investigating the role of Cu^{2+} on reproductive health displayed conflicted results; in infertility and preeclampsia, both increased and decreased levels are reported [11, 12].

Thiols are functional –SH (sulfur and hydrogen atom) groups that reside in the cysteine structure (-S-S). Total thiols can either take part in glutathione as free form or be bound to cysteine groups of proteins. Even the cysteine groups only correspond to 3% of whole protein, they are crucial for proteins for their pleiotropic functions on signal transduction, molecular stabilization, forming metal complexes and antioxidant defense as a potent reductant [13]. Thiol pool term corresponds to the richness of these cysteine groups in the related compartment. In the context of plasma thiol levels, relatively lower amounts are present, and these are found mainly in the human serum albumin (HSA) structure [13]. In the last 20 years, this topic took great attention of researchers and numerous studies found disruptive thiol/disulfide homeostasis in multiple diseases. Thiol/disulfide homeostasis is used as a novel marker for oxidative research, and the shift in favor of the oxidized form, disulfide, has been found to be associated with the increased oxidative status. According to this, reduction in the thiol pool will lead redox imbalance and constitute for variety of inflammatory disease processes and as one of them is broadly discussed here, preeclampsia. Our study examines total-native thiol levels with disulfide levels of preeclamptic and normotensive pregnant women. Correlation analysis of thiol levels with zinc and copper levels also will be performed.

The aim of our current study is to investigate the levels of zinc and copper in the serums of pregnant women with or without preeclampsia. As a well-established concept, oxidative stress plays an important etiological role in the development of preeclampsia. We evaluate the thiol/disulfide status in two groups as a novel oxidative stress marker and also look for a possible correlation between thiol levels and the investigated parameters. The study will provide new evidence and contribute to the efforts for understanding preeclampsia by reviewing previous studies and discussing the role of trace elements in the pathogenesis.

Materials and Methods

This observational case-control study was planned to compare the serum levels of zinc and copper between preeclampsia and normotensive pregnant women. Element measurements were done in all volunteers that were in their third trimester pregnancy (from week 28 to week 40). The study complies with the Declaration of Helsinki and was approved by the clinical research ethics committee of Bezmialem Vakıf University (Ethical number: 18/352, 27.10.2020). Each volunteer has been asked to provide written informed consent. The preeclampsia patients had been diagnosed according to the diagnostic criteria of American College of Obstetricians and Gynecologists ACOG Practice Bulletin No. 202: Gestational Hypertension and Preeclampsia [14] and followed until their delivery date in the obstetrics and gynecology clinic of Istanbul Education Research Hospital.

Population of Study

Power analysis with 80% power level revealed that a minimum number of 42 individuals for each group (total number

of 84) should be included in the study (given the effect size of 0.62 and p value of 0.05). From September 2020 to December 2020, our case-control study consisted of 43 preeclamptic women and 45 normotensive healthy pregnant women as a control group. All individuals participated in the study were selected according to the following inclusion criteria and excluded if they met one or more of the following exclusion criteria.

Inclusion Criteria

Pregnant women aged in 18–45 and in their third trimester pregnancy were selected, and preeclampsia diagnosis has been made if normally normotensive pregnant woman has ≥ 140/90 mmHg systolic blood pressure (in repeated measurements with 6-h interval) and proteinuria (24-h urine collection, ≥ 300 mg) after 20th gestational week.

Exclusion Criteria

Maternal diabetes, obesity (body mass index > 30 kg/m^2), chronic diseases before pregnancy, pathologies leading to hypertension and proteinuria rather than preeclampsia, malignancies, cardiovascular and renal diseases, drug usage, genetic diseases, alcohol and cigarette usage, recent history of surgery or transfusion, having treatment with medications, fetal anomalies, fetal death in utero, active infection.

Sample Preparation

Blood samples were collected in obstetrics and gynecology clinic of Istanbul Education Research Hospital following 12 h of fasting from the cubital vein to biochemistry tubes that do not contain separation gel (for trace element analysis). The serums were separated after centrifugation. Samples were then analyzed in Bezmialem University routine clinical laboratory.

Methods

Serum zinc concentrations were measured with commercially available Rel Assay zinc measurement kit (Gaziantep, Turkey) using a fully automatic photometric method (Abbott ARCHITECT c8000 clinical chemistry analyzer). The principle of the Zn^{2+} method is that total zinc in the sample changes the red-orange color of 5-Br-PAPS to light pink in alkaline conditions. Absorbance change measured at 548 nm is proportional to the concentration of Zn^{2+} in the sample (μg/dL). The standard of the method is zinc sulfate.

Serum copper concentrations were measured with commercially available Rel Assay copper measurement kit (Gaziantep, Turkey), using a fully automatic photometric method (Abbott ARCHITECT c8000 clinical chemistry analyzer). The novel method principle depends on the color change of red-orange DiBr-PAESA to violet in alkaline conditions that is proportional to the copper concentration in samples (μg/dL). Absorbance change is measured at 572 nm, and copper sulfate is used as a standard.

Serum levels of total and native thiol were measured with a novel method developed by Erel and Neselioglu [15]. In this method, serum dynamic disulfide bonds (-S-S-) is reduced to native thiol groups (-SH HS-) by sodium borohydride ($NaBH_4$) and native thiol levels were measured. Total thiol levels (μmol/L) were measured using a fully automatic photometric method (Abbott ARCHITECT c8000 clinical chemistry analyzer) using Ellman reagent. The disulfide level is calculated by subtracting the native thiol level from the total thiol level and taking half of it.

Serum ALT and AST levels were measured by using a fully automatic photometric method (Abbott ALT/AST kit, Abbott ARCHITECT c8000 clinical chemistry analyzer).

Serum urea (urea nitrogen) and creatinine levels were measured by using a fully automatic photometric method (Abbott urea nitrogen/ creatinine analyte kit, Abbott ARCHITECT c8000 clinical chemistry analyzer).

Statistical Analysis

The G Power analysis program was used to calculate the sample size of the study. Data distributions were assessed with a single sample Kolmogorov-Smirnov test and were found normally distributed ($p \geq 0.05$). Results were analyzed using independent sample t-test to determine any statistically significant difference between the groups, and MANOVA test was applied for matching groups. Data were expressed as the mean ± standard deviation (SD) in Table 1, and p value less than 0.05 was considered statistically significant. Receiver operating characteristic curve (ROC) analysis was carried out to define optimum cutoff values of copper and copper/native thiol (Cu^{2+}/NThiol ratio) for the prediction of preeclampsia. Discriminant analysis was employed for the Cu^{2+}/NThiol ratio to assess its segregation capacity for PE patients and controls. All parameters were included initially, and stepwise discriminant analysis was followed to identify each parameter's function coefficients. Analyses were done with IBM SPSS version 27.0 software (IBM Corporation, Armonk, NY, USA).

Results

Sociodemographic Data

The sociodemographic parameters of the individuals are presented in Table 1. There was no significant age, gestational age (weeks), and BMI differences between the patient and control groups ($p > 0.05$). Aspartate transaminase (AST) and

Table 1 Demographical and clinical characteristics of preeclampsia patients and healthy pregnant women. Values are shown as mean ± standard deviation (ns: not significant; *0.01 < p < 0.05; **0.001 < p < 0.01; ***p < 0.001 level of significance of effects)

Parameter	Control	Preeclampsia	p value
Age	27.2 ± 5.3	30.2 ± 6.8	ns
BMI	29.8 ± 3.9	29.5 ± 5.5	ns
Gestational week	35.8 ± 1.9	35.3 ± 3.3	ns
Cu^{2+}	195.2 ± 38.6	224.5 ± 29.6	0.001***
Zn^{2+}	80.3 ± 17.7	75.1 ± 20.9	ns
NThiol	214.2 ± 52.9	137.8 ± 28.2	0.000***
TThiol	429.5 ± 95.6	305.8 ± 73.9	0.000***
Disulfide	107.7 ± 21.4	84 ± 17.5	ns
ALT	12.9 ± 4.2	16.8 ± 7.4	0.011*
AST	11.9 ± 5.1	18.9 ± 10.9	0.001***
Urea	12.4 ± 9.6	17.7 ± 8.2	ns
Creatinin	0.4 ± 0.1	0.5 ± 0.1	ns
Cu^{2+}/Zn^{2+}	2.4 ± 0.8	3.2 ± 0.9	0.001***

alanine aminotransferase (ALT) mean values were close to the upper limit of the normal reference range in the preeclamptic patients with the values of 18.9 and 16.8 units per liter (IU/L), respectively (Table 1). ALT and AST levels were significantly different in two groups (p < 0.05).

Trace Element Status

Assessing the zinc status of 43 preeclamptic and 45 healthy pregnant women, zinc level is found slightly decreased in both groups, with the mean value of 80.3 μg/dL in controls and 75.1 μg/dL in patients. Considering that 66 to 110 μg/dL of normal zinc values in healthy adults drops to 50–77 μg/dL in the third trimester (in healthy pregnant women), our groups did not show an important decrease (p > 0.05)[16]. There was also no significant difference in zinc levels comparing two groups (p > 0.05). Conversely, the copper level is increased physiologically in pregnancy and normal reference interval for the third trimester is 130–240 μg/dL [16]. Even an increased copper levels have been found in both groups, preeclamptic patients had significantly higher levels with the mean value of 224.5 μg/dL than controls, with the mean value of 195.2 μg/dL (p < 0.05). These findings are shown in Table 1.

Figure 1 shows ROC analysis of copper levels and Cu^{2+}/NThiol ratio. AUC values were 0.72 for copper and 0.90 for Cu^{2+}/NThiol ratio (Table 2). The cutoff values were 224 μg/dL for copper and 1.19 for Cu^{2+}/NThiol ratio, and these values predicted preeclampsia with 59% and 86% sensitivity with 78% and 90% specificity, respectively (95% CI 0.85–0.99 for copper and 0.82–0.99 for Cu^{2+}/NThiol, p < 0.001) (as shown in Table 2). Figure 2 shows that Cu^{2+}/NThiol ratio was significantly different between the two groups (p < 0.05). Discriminant analysis revealed that when combined with AST levels, Cu^{2+}/ NThiol ratio significantly separated PE patients from controls with an overall accuracy of 76.5% (67.4% sensitivity and 90.0% specificity, Table 3). Table 4 shows the function coefficients of the two clinical markers, Cu^{2+}/Nthiol ratio and AST. Depending on these

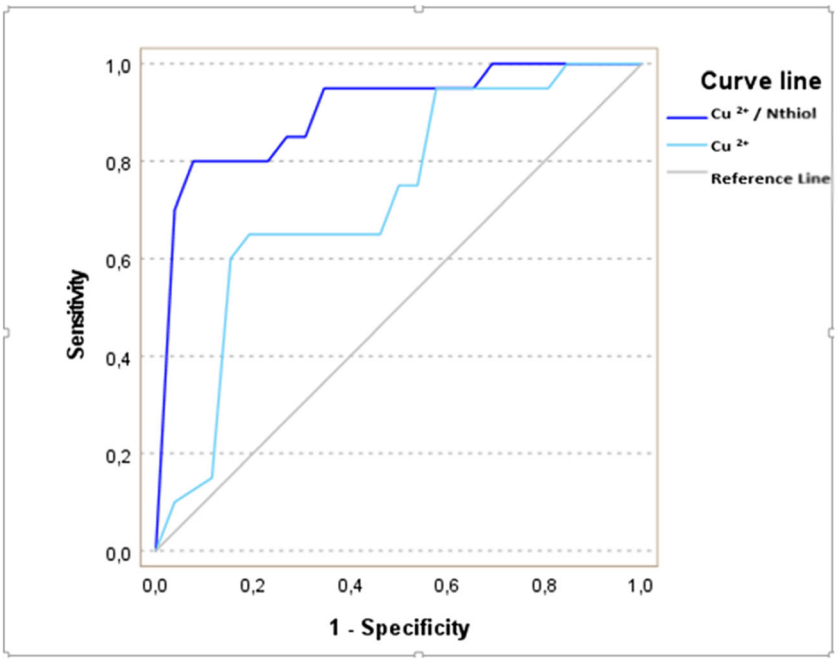

Fig. 1 ROC analysis for copper and Cu^{2+}/NThiol ratio in the prediction of preeclampsia

Table 2 AUC values of copper and Cu^{2+}/NThiol ratio

Area under the curve					
Test result variable(s)	Area	Std. error	Sig.	Asymptotic 95% confidence interval	
				Lower bound	Upper bound
Cu^{2+}	.72	.08	.004	.59	.85
Cu^{2+}/NThiol	.90	.04	.000	.82	.99

values, the equation of segregation function was determined.

Assessment of the relationship between the two trace element for both groups revealed a positive correlation in healthy pregnant women ($p < 0.05$, $r = 0.43$, as shown in Table 5). There was no significant correlation between element levels for preeclamptic patients ($p > 0.05$, Table 6).

Total and Native Thiol Levels

Table 1 reports total-native thiol status of preeclampsia patients and healthy pregnant women. Total and native thiol levels were significantly lower in preeclampsia patients than the control group with the mean values of 305.8 μmol/L and 137.8 μmol/L, respectively ($p < 0.05$). The correlation analysis between zinc and total thiol levels revealed a strong positive relationship in healthy controls ($p < 0.05$, $r = 0.64$, Table 5), while this relationship was disrupted in preeclampsia patients (as shown in Table 6). The calculated disulfide levels were not statistically different between the two groups with the mean values of 107.7 μmol/L in controls and 84 μmol/L in patients ($p > 0.05$, Table 1).

Discussion

As main findings of the present study, we found a significant increase in serum copper levels with a predictive cutoff value of 224 μg/dL (AUC value of 0.72) and in Cu^{2+}/ NThiol ratio with a predictive value of 1.19 (AUC value of 0.90). Copper to native thiol ratio had an outstanding ability to diagnose patients, and we assume that this ratio is the best predictor of PE according to the present literature. As discriminant analysis revealed, combining AST levels with the Cu^{2+}/NThiol ratio had correctly predicted PE patients with overall accuracy of 76.5%. Decreased levels of total thiol in patients were also found compared to controls. Disulfide levels were not statistically different between the groups. A slight decrease in zinc serum levels was not significantly different between the two groups. ALT and AST levels were marginally high and significantly higher in the preeclampsia group. As another recent finding, an impaired association of Zn^{2+}- Cu^{2+} levels was found in preeclampsia patients that are normally seen as a negative correlation in healthy people. Our study is also first to examine the relationship between zinc and total thiol levels in preeclampsia patients, and a disrupted relationship was found in patients while the control group showed a strong positive correlation.

Investigating zinc and copper metabolism in preeclamptic patients revealed a possible relationship but with the contradictory results. Several studies have found significantly lower zinc and copper levels in preeclamptic women compared to controls [12, 17]. One of these studies displayed significantly increased SOD enzyme activity as the compensator factor for lower Zn^{2+}-Cu^{2+} in patient group [12]. Elevated levels of Cu^{2+} with decreased levels of Zn^{2+} have been reported, and these findings coincides with the current study [18]. Comparing element levels in mild and severe preeclampsia, few studies found increased levels of copper as an indicator of oxidative

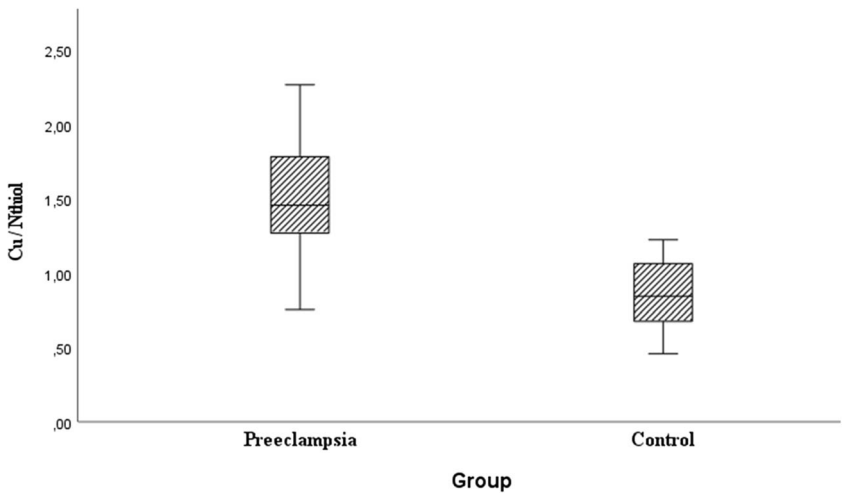

Fig. 2 Cu^{2+}/NThiol ratio shows a significant difference between the two groups ($p < 005$)

Table 3 Classification results show that when combined with AST levels, Cu^{2+}/Nthiol ratio had 67.4% sensitivity and 90.0% specificity with overall accuracy of 76.5% for predicting PE

Classification results[a]			
Group	Preeclampsia	Control	Total
Preeclampsia	29	14	43
Control	4	40	45
Preeclampsia	67.4	34.6	100
Control	10	90	100

[a] About 76.5% of original grouped cases correctly classified

Table 4 Function coefficients of the two clinical marker (Cu^{2+}/Nthiol ratio and AST)

Classification function coefficients		
Parameter	Preeclampsia	Control
Cu^{2+}/Nthiol	18.6	11.2
AST	.48	.29
(Constant)	− 18.3	− 7.2

stress together with increased lipid peroxides in severe preeclamptic patients [19, 20]. A 2010 study concluded that zinc serum levels were significantly lower in both mild and severe preeclampsia compared to controls, but there was no significant difference between the two patient groups [7]. Contradictory to these findings, a study revealed increased levels of zinc in preeclamptic women compared to controls [21]. An insignificant difference in zinc levels in patient and control group has also been reported [22].

As the previous studies put forth, zinc deficiency appears to be strongly associated with preeclampsia pathophysiology and may be a risk factor for the development of the disease, although there are some studies found elevated levels in contrast. Our finding of lower but not significantly different Zn^{2+} levels in patients agrees with the major part of previous studies on the issue [3, 23]. Lacking Zn^{2+} may promote the oxidative stress by increasing the lipid peroxidation due to decreased antioxidant defense and with the insufficiency of zinc dependent antioxidant enzymes such as Cu-Zn SOD [24]. Further, important knowledge of the relationship between zinc deficiency and preeclampsia, fetal growth restriction, prematurity, birth defects, and immune abnormalities is supporting the involvement of Zn^{2+} in ensuring physiological pregnancy process [25]. However, decreased Zn^{2+} concentrations observed in preeclamptic patients may be an outcome of the albumin loss which is a major component of proteinuria seen in preeclampsia. Albumin is a main transporter of Zn^{2+} in the plasma and utilizes zinc delivery to cells; its loss will eventually lead decreased total Zn^{2+} levels, a possible explanation for our finding of marginally lower Zn^{2+} levels [26]. Even more important mechanism, activated inflammatory processes in preeclampsia pathogenesis may contribute to the deficient element state [27, 28]. Suggestions for preventing the poor maternal and fetal outcomes with early supplementation of zinc appears clinically valuable [29]. Zn^{2+} supplementation for preventing preeclampsia is still up for debate, and further studies with larger sample size are needed to reach the final outcome.

Copper plasma levels in preeclamptic patients displayed more heterogenic pattern and this fact may be due to the Cu^{2+}'s complex actions in the organisms mediating both oxidative and antioxidative pathways. Our current work found significantly elevated serum levels of Cu^{2+} in preeclampsia patients than healthy pregnant women, and this finding is consistent with the other several publications [18–20]. The ROC analysis revealed Cu^{2+}'s predictive cutoff value as 224 μg/dL and AUC value of 0.72, indicating that Cu^{2+} may be assumed as an acceptable biomarker for PE diagnosis.

During pregnancy, copper levels are physiologically increased and each trimester has its own normal ranges [16]. This fact may be the result of high amounts of serum estrogens in pregnant women considering that previous works proved increased serum copper levels by estradiol therapy or in pregnancy period [30, 31]. The mechanism may be explained by increased expression of ceruloplasmin (Cp) under estrogenic activity since estrogens are one of the main determinants of Cp expression [32]. Although preeclampsia patients displayed lower estradiol concentrations [33], they had elevated Cp levels which may clarify higher copper levels compared to controls [34]. On the other part, a considerable amount of evidence proves the copper's role in the activated oxidative response and its elevated levels are associated with development of many pathological processes [35]. Since elevated lipid peroxidation and its products (MDA) have been reported in preeclampsia patients [12, 18–20], Cu^{2+}, as a transition metal, may contribute to disease progression by producing free radicals that lead to lipid peroxidation and damaging endothelial cell function. Other supporting data strengthening the oxidative imbalance induced by copper hypothesis came from several studies that showed elevated ceruloplasmin levels since Cp is a potent antioxidant molecule itself, the increase of it may be recognized as a compensation response for the elevated copper levels and oxidative stress [36]. Ceruloplasmin was not included in the current study as elevated Cp levels in PE have been shown by numerous studies [19, 34]. Another robust theory may explain the elevated copper levels as it results from an activated inflammatory response other than being an etiological factor [37]. Cu^{2+} elevation in serum may also be dependent on the Zn^{2+} decrease considering the antagonism between the two elements [38]. According to our results, increased copper levels in patients contribute to oxidative damage and so are related to the preeclampsia pathogenesis. Although this premise agrees with multiple studies, there are contradictory results. So, copper levels in preeclampsia patients should be analyzed together with parameters that affect

Table 5 Correlation table of the parameters showing the significant relationships observed in the control group

Pearson correlation	Cu^{2+} (μg/dL)	Zn^{2+} (μg/dL)	NThiol (μmol/L)	TThiol (μmol/L)	*p* value
Cu^{2+} (μg/dL)		0.43			0.014
Zn^{2+}(μg/dL)				0.64	0.000
NThiol (μmol/L)					
TThiol (μmol/L)					

Cu^{2+} metabolism like other elements and serum proteins. In addition, antioxidant supplementation would be in great value for both preventing increased oxidative damage by copper excess and reverse the disadvantage of lower levels of an important antioxidant element, zinc, in preeclampsia patients.

Other finding of the study is positively correlated Zn^{2+} and Cu^{2+} levels in normotensive pregnant women. However, this association was found impaired in preeclampsia patients. Physiologically increased copper levels in healthy pregnant women may be the reason for positively correlated element levels as our current study showed. This association was not obtained for patient group and the finding may indicate imbalanced trace element metabolism in preeclampsia.

The finding of significantly higher ALT and AST levels in patients refer to damaged liver functions seen in preeclampsia patients [39]. Researchers showed elevated levels of these enzymes in the serums of patients and they suggested its use as a prediction tool for both severity of disease and adverse materno-fetal outcomes [39]. Current results displayed significant difference between groups, but the mean values were in the normal reference range. Since previous works majorly found abnormal high AST and ALT levels in patients, further studies are needed to confirm our findings.

The total and native thiol levels were found significantly decreased in preeclampsia patients compared to control group. This decrease may be an outcome of promoted oxidative pathways observed in the disease progress so that antioxidant effectors such as thiols will be diminished to buffer the oxidative stress [40]. Besides, proteinuria in preeclampsia results in albumin loss and considering that albumin is the main thiol group carrier protein in the plasma, total and native thiol levels will secondarily drop off in accordance with our findings in this study. Previous studies have confirmed the declined thiol levels in preeclampsia [41, 42]. Our study is first to assess the correlation between total thiol and zinc levels in patients with healthy controls. A positive strong correlation that exists in the normotensive pregnant women changed in the patient group as no correlation was found.

Serum disulfide levels correspond to disulfide bonds that reside in the cysteine groups of serum proteins (mainly albumin). In the presence of increased oxygen radicals, reduced thiol groups turn into oxidized state and form disulfide bonds [43]. Therefore, increased serum disulfide levels may reflect redox imbalance in favor of oxidative processes. Both total and native thiol concentrations were found decreased in patients while disulfide levels were not statistically different between the groups. Patients have been expected to have higher disulfide levels due to the promoted oxidative stress seen in preeclampsia pathogenesis [44]. However, since disulfide levels are not directly measured and calculated from total-native thiol levels, the estimated numbers may not reflect the exact levels and be dependent on the sample size. Hence, our finding of not different levels may be due to the limited number of individuals enrolled in the study. Larger sample sized trials are needed to reveal more reliable results for serum disulfide levels.

Except from these, the most important contribution of our study was the finding of elevated Cu^{2+}/NThiol ratio which appears as an outstanding predictor of PE. As far as we know, this ratio had the highest diagnostic value for PE according to our ROC analysis. Due to increased levels of copper and loss of thiol containing proteins with proteinuria, the ratio was significantly higher in patients than healthy pregnant women. We offer that this novel parameter may be used as an early indicator of preeclampsia. To test this premise, we also call for further studies investigating the ratio in high risk population before the onset of disease.

Conclusion

In conclusion, our present work shows that preeclampsia patients have considerably lower serum levels of zinc and

Table 6 Correlation table of the parameters showing the significant relationships observed in the patient group

Pearson correlation	Cu^{2+} (μg/dL)	Zn^{2+} (μg/dL)	NThiol (μmol/L)	TThiol (μmol/L)	*p* value
Cu^{2+} (μg/dL)		0.24			0.146
Zn^{2+}(μg/dL)				0.06	0.729
NThiol (μmol/L)					
TThiol (μmol/L)					

significantly higher levels of copper compared to normotensive healthy pregnant women. Crucial finding about elevated copper levels in patients contributes to the literature which has heterogeneous results. Oxidative markers, total and native thiol levels with disulfide levels were also investigated. Total-native thiol levels have been found lower in preeclamptic group as consistent with the previous study findings while disulfide levels were not statistically different between the groups. Altogether, the most important finding was the elevated Cu^{2+}/NThiol ratio in PE patients with an outstanding diagnostic value. We offer that this novel parameter may be used as an indicator of PE development. Still, its predictive role is an inquiry that needs to be investigated with further studies. Moreover, considering both elevated oxidative damage conducted by excess copper and decreased antioxidant defense due to zinc and thiol deficiency, antioxidant supplementation may be beneficial for reversing the altered oxidative status in preeclampsia patients and also may help for preventing the development of PE in predisposed healthy pregnant women.

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Author Contribution Ayse Zehra Gul, Nil Atakul, and Sahabettin Selek contributed to the research equally.

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Data Availability Based on a request, the data supporting the current work's findings is available from the corresponding author.

Code Availability Based on a request, the code is available from the corresponding author.

Declarations

Ethics Approval This research was conducted in accordance with the Declaration of Helsinki and approved by the clinical research ethics committee of Bezmialem Vakıf University (No. 54022451-050.05.04).

Consent to Participate Each subject was informed about the study purpose, and a written consent was obtained from all participants.

Consent for Publication Approvals from all authors were obtained for publication.

Conflict of Interest The authors declare that they have no conflict of interest.

References

1. Bartsch E, Medcalf KE, Park AL, Ray JG (2016) Clinical risk factors for pre-eclampsia determined in early pregnancy: systematic review and meta-analysis of large cohort studies. Bmj 353:i1753
2. August P, Sibai BM (2017) Preeclampsia: Clinical features and diagnosis. Post TW, UpToDate Waltham, MA: UpToDate
3. Sarwar MS, Ahmed S, Ullah MS, Kabir H, Rahman GM, Hasnat A, Islam MS (2013) Comparative study of serum zinc, copper, manganese, and iron in preeclamptic pregnant women. Biol Trace Elem Res 154(1):14–20
4. Weber D, Stuetz W, Bernhard W, Franz A, Raith M, Grune T, Breusing N (2014) Oxidative stress markers and micronutrients in maternal and cord blood in relation to neonatal outcome. Eur J Clin Nutr 68(2):215–222
5. Mills CF (2013) Zinc in human biology. Springer Science & Business Media,
6. Hambidge KM, Krebs NF (2007) Zinc deficiency: a special challenge. J Nutr 137(4):1101–1105
7. Jain S, Sharma P, Kulshreshtha S, Mohan G, Singh S, Bter J (2010) The role of calcium, magnesium, and zinc in pre-eclampsia. Biol Trace Elem Res 133(2):162–170
8. Karim N (2018) Copper and Human Health-A Review. J Bahria Univ Med Dental Coll 8(2):117–122
9. Gambling L, Kennedy C, McArdle HJ (2011) Iron and copper in fetal development. In: Seminars in cell & developmental biology. vol 6. Elsevier, pp 637-644
10. Bost M, Houdart S, Oberli M, Kalonji E, Huneau J-F, Margaritis I (2016) Dietary copper and human health: Current evidence and unresolved issues. J Trace Elem Med Biol 35:107–115
11. Bawa R, Tyagi S (2017) Correlation of microelements like plasma copper and zinc concentrations with female infertility. Int J Reprod Contracept Obs Gynecol 6:2351–2353
12. Keshavarz P, Gh BFNM, Mirhafez SR, Nematy M, Azimi-Nezhad M, Afin SA, Esmaily H, Pourali L, Hakak AM, Soukhtanloo M (2017) Alterations in lipid profile, zinc and copper levels and superoxide dismutase activities in normal pregnancy and preeclampsia. Am J Med Sci 353(6):552–558
13. Turell L, Radi R, Alvarez B (2013) The thiol pool in human plasma: the central contribution of albumin to redox processes. Free Radic Biol Med 65:244–253
14. Practice Bulletin No AJOG (2019) 202: gestational hypertension and preeclampsia. Obstet Gynecol 133(1):e1–e25
15. Erel O, Neselioglu S (2014) A novel and automated assay for thiol/disulphide homeostasis. Clin Biochem 47(18):326–332
16. Abbassi-Ghanavati M, Greer LG, Cunningham FG (2009) Pregnancy and laboratory studies: a reference table for clinicians. Obstet Gynecol 114(6):1326–1331
17. Akinloye O, Oyewale O, Oguntibeju OO (2010) Evaluation of trace elements in pregnant women with pre-eclampsia. Afr J Biotechnol 9(32):5196–5202
18. Atamer Y, Koçyigit Y, Yokus B, Atamer A, Erden AC (2005) Lipid peroxidation, antioxidant defense, status of trace metals and leptin levels in preeclampsia. Eur J Obstet Gynecol Reprod Biol 119(1): 60–66
19. Serdar Z, Gür E, Develioğlu O (2006) Serum iron and copper status and oxidative stress in severe and mild preeclampsia. Cell Biochem Funct: Cellular biochemistry and its modulation by active agents or disease 24(3):209–215
20. Rafeeinia A, Tabandeh A, Khajeniazi S, Marjani AJ (2014) Serum copper, zinc and lipid peroxidation in pregnant women with pre-eclampsia in gorgan. Open Biochem J 8:83
21. Harma M, Harma M, Kocyigit A (2005) Correlation between maternal plasma homocysteine and zinc levels in preeclamptic women. Biol Trace Elem Res 104(2):97–105
22. Vafaei H, Dalili M, Hashemi SA (2015) Serum concentration of calcium, magnesium and zinc in normotensive versus preeclampsia pregnant women: a descriptive study in women of Kerman province of Iran. Iran J Reproduct Med 13(1):23–26
23. Elmugabil A, Hamdan HZ, Elsheikh AE, Rayis DA, Adam I, Gasim GI (2016) Serum calcium, magnesium, zinc and copper

levels in sudanese women with preeclampsia. PLoS One 11(12): e0167495
24. Acikgoz S, Harma M, Harma M, Mungan G, Can M, Demirtas S (2006) Comparison of angiotensin-converting enzyme, malonaldehyde, zinc, and copper levels in preeclampsia. Biol Trace Elem Res 113(1):1–8
25. Gzhegotskyi M, Sukhodolska N (2019) Lead, cadmium, copper and zinc content in women's blood during the third trimester of uncomplicated and complicated gestation. Exp Clin Physiol Biochem 2019:5–11. https://doi.org/10.25040/ecpb2019.03.005
26. Kumera G, Awoke T, Melese T, Eshetie S, Mekuria G, Mekonnen F, Ewunetu T, Gedle D (2015) Prevalence of zinc deficiency and its association with dietary, serum albumin and intestinal parasitic infection among pregnant women attending antenatal care at the University of Gondar Hospital, Gondar, Northwest Ethiopia. BMC Nutr 1(1):31
27. Gammoh NZ, Rink L (2017) Zinc in infection and inflammation. Nutrients 9(6):624
28. Olechnowicz J, Tinkov A, Skalny A, Suliburska J (2018) Zinc status is associated with inflammation, oxidative stress, lipid, and glucose metabolism. J Physiol Sci 68(1):19–31
29. Ota E, Mori R, Middleton P, Tobe-Gai R, Mahomed K, Miyazaki C, Bhutta ZA (2015) Zinc supplementation for improving pregnancy and infant outcome. Cochrane Database Syst Rev (2)
30. Ulas M, Cay M (2011) Effects of 17β-estradiol and vitamin E treatments on blood trace element and antioxidant enzyme levels in ovariectomized rats. Biol Trace Elem Res 139(3):347–355
31. Bednarek-Tupikowska G, Jodkowska A, Antonowicz-Juchniewicz J (2010) Zinc, cooper, manganese, and selenium status in pre-and postmenopausal women during sex hormone therapy Stężenia cynku, miedzi, manganu i selenu u kobiet menopauzalnych przyjmujących terapię hormonalną. Adv Clin Exp Med 19(3): 337–345
32. Linder M (2016) Ceruloplasmin and other copper binding components of blood plasma and their functions: an update. Metallomics 8(9):887–905
33. Cantonwine DE, McElrath TF, Trabert B, Xu X, Sampson J, Roberts JM, Hoover RN, Troisi R (2019) Estrogen metabolism pathways in preeclampsia and normal pregnancy. Steroids 144:8–14
34. Bellos I, Papantoniou N, Pergialiotis V (2018) Serum ceruloplasmin levels in preeclampsia: a meta-analysis. J Matern Fetal Neonatal Med 31(17):2342–2348
35. Uriu-Adams JY, Keen CL (2005) Copper, oxidative stress, and human health. Mol Asp Med 26(4-5):268–298
36. Nikolic A, Cabarkapa V, Novakov Mikic A, Jakovljević A, Stosic Z (2016) Ceruloplasmin and antioxidative enzymes in pre-eclampsia. J Matern Fetal Neonatal Med 29(18):2987–2993
37. Sorenson JR (2012) Inflammatory diseases and copper: the metabolic and therapeutic roles of copper and other essential metalloelements in humans, vol 2. Springer Science & Business Media,
38. Nishito Y, Kambe T (2018) Absorption mechanisms of iron, copper, and zinc: an overview. J Nutr Sci Vitaminol 64(1):1–7
39. Alese MO, Moodley J, Naicker T (2019) Preeclampsia and HELLP syndrome, the role of the liver. J Matern Fetal Neonatal Med 34(1): 117–123 1-7
40. Ghezzi P, Bonetto V, Fratelli M (2005) Thiol–disulfide balance: from the concept of oxidative stress to that of redox regulation. Antioxid Redox Signal 7(7-8):964–972
41. Korkmaz V, Kurdoglu Z, Alisik M, Cetin O, Korkmaz H, Surer H, Erel O (2016) Impairment of thiol-disulfide homeostasis in preeclampsia. J Matern Fetal Neonatal Med 29(23):3848–3853
42. Ozler S, Erel O, Oztas E, Ersoy AO, Ergin M, Sucak A, Neselioglu S, Uygur D, Danisman N (2015) Serum thiol/disulphide homeostasis in preeclampsia. Hypertens Pregnancy 34(4):474–485
43. Cremers CM, Jakob U (2013) Oxidant sensing by reversible disulfide bond formation. J Biol Chem 288(37):26489–26496
44. Onat T, Aydoğan Kırmızı D, Başer E, Ercan M, Demir Çaltekin M, Yalçın S, Kara M, Esinler D, Yalvaç ES (2020) The relationship between oxidative stress and preeclampsia. The serum ischemia-modified albumin levels and thiol/disulfide homeostasis. Turk J Obstet Gynecol 17(2):102–107. https://doi.org/10.4274/tjod.galenos.2020.23682