RESEARCH ARTICLE

Lower Ferrum, Selenium, and Cadmium; Higher Chromium and Lead Levels in Preeclampsia Patients' Erythrocyte: A Cross-Sectional Study

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Abstract

BACKGROUND: Oxidative stress and trace elements in erythrocytes are linked to impaired nitric oxide that can lead to endothelial dysfunction in preeclampsia patients. The morphology of erythrocytes could also be affected by oxidative stress and trace elements. While the relationships between erythrocyte index, superoxide dismutase (SOD) activity, and oxidative stress in preeclampsia have been well established, less attention has been given to the erythrocyte trace elements and their role in disease progression. This study was performed to examine the erythrocyte trace element profile in women with preeclampsia, comparing it with controls. Additionally, it will explore the correlations between erythrocyte trace element levels, the erythrocyte index, and SOD activity.

METHODS: A cross-sectional study was conducted involving 40 pregnant women consisting of those with severe preeclampsia and normotensive. Erythrocytes was isolated from blood samples, and analysis of erythrocyte SOD activity and trace elements were performed using the enzyme linked immunosorbent assay (ELISA) and inductively coupled plasma mass spectrometry (ICP-MS), respectively.

RESULTS: Among 15 examined erythrocyte trace elements, the levels of ferrum (Fe), selenium (Se), and cadmium (Cd) were significantly lower, meanwhile, the levels of chromium (Cr) and lead (Pb) were significantly higher in preeclampsia subjects. Additionally, preeclampsia subjects exhibited smaller erythrocyte sizes compared to the normotensive subjects. The erythrocyte SOD activity was significantly elevated in the preeclampsia subjects than the normotensive subjects.

CONCLUSION: Erythrocyte trace element levels of Fe, Se, Cd, Cr, and Pb were significantly altered in preeclampsia compared to normotensive controls. These findings suggest that these trace elements may serve as potential predictors for preeclampsia.

KEYWORDS: preeclampsia, trace elements, antioxidant, oxidative stress, superoxide dismutase, erythrocyte index

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Introduction

Preeclampsia is a major etiology of maternal and infant morbidity and mortality worldwide. Based on the 2018 Basic Health Research in Indonesia, hypertension in pregnancy is one of the three leading causes of maternal morbidity and mortality, with a prevalence of around 2.7%. (1) Until now, existing studies have not been able to fully reveal the etiology and pathogenesis of preeclampsia.(2)

The pathophysiology of preeclampsia is closely related to oxidative stress. It is generally recognized that oxidative stress occurs in the placenta, trophoblast, or vascular endothelium.(3,4) Abnormality was found in trophoblast

invasion to myometrium and endothelial dysfunction in placenta, then spreads to maternal circulation.(5) However, increased oxidative stress can also occur in plasma and erythrocytes.(6) Maternal erythrocytes can play a role in causing maternal endothelial dysfunction through impaired nitric oxide (NO) balance.(7) Co-incubation of mice's aorta with erythrocytes of women with preeclampsia can induce endothelial dysfunction. However, this event did not occur in the erythrocytes of healthy pregnant women. This suggests a role for erythrocytes in endothelial dysfunction in preeclampsia patients.(7)

The main antioxidant systems in erythrocytes are superoxide dismutase (SOD), glutathione, glutathione peroxidase, glutathione S-transferase, glutathione reductase, and catalase.(8) These antioxidant enzymes need trace elements such as selenium (Se), cuprum (Cu), zinc (Zn), manganese (Mn), and ferrum (Fe) as co-factors for the practical function of these enzymes. Oxidative stress and trace elements in erythrocytes are essential in causing impaired NO production.(8–11)

A recent literature study demonstrated the importance of cellular wellness and nutrition in the incidence of preeclampsia.(12) Maternal erythrocytes are important in causing maternal endothelial dysfunction via oxidative stress and trace elements. Until now, there is still no study that evaluates the erythrocyte index, SOD activity, and trace elements even though it is important to understand the status of these parameters between preeclampsia and normotensive pregnant women. Therefore, this study was performed to examine the erythrocyte trace element profile in women with preeclampsia and comparing it with controls. The results of this study might help finding the best potential predictors for preeclampsia.

Methods

Study Design and Subjects Recruitment

A cross-sectional study was conducted in Dr. Cipto Mangunkusumo National Referral Hospital, Tangerang Regional Hospital, and Koja Region Hospital from October 2020 to March 2021. The total subjects included in the study was 40 pregnant women which were classified into normotensive (n=20) and preeclampsia with severe features (n=20). Patients with a gestational age of 20 weeks to term that fulfill the criteria of preeclampsia with severe features based on the American College of Obstetricians and Gynecologists (ACOG) criteria were included in this study.(13) Pregnant subjects with diabetes mellitus, heart disease, hemoglobinopathy, autoimmune disease, or congenital anomalies were excluded from the study. The protocol of the study has been approved by the Ethical Committee for Research in Human from the Faculty of Medicine, Universitas Indonesia (No. KET—236/UN2.F1/ ETIK/ PPM.00.02/2021).

Sample Preparation and Erythrocyte Isolation

Blood samples were collected using venous puncture into several tubes; 3 mL blood into EDTA tube, 6 mL blood into sodium heparin containing tube, and 6 mL blood into plain tube for various analysis. Initially, erythrocyte should be isolated for the analysis of erythrocyte SOD activity and trace elements. First, 2 mL of blood sample was pipetted into a polypropylene tube with 2 mL of PBS and homogenized. Then 3 mL of Ficoll-Paque Plus solution was added, homogenized, and the sample was centrifuged at 400 g for 30 minutes at 19°C. After forming the erythrocyte layer and supernatant, supernatant layer was removed, and 2 mL PBS was added. The erythrocytes part was then washed 3 times until cleaned by centrifugation at 100 g for 10 minutes. After the last washing step, phosphate buffered saline (PBS) was added to the final volume of 2 mL, and then the erythrocyte sample was ready to use.

Erythrocyte Indices Measurement

Erythrocytes indices, including hemoglobin, hematocrit, erythrocyte concentration, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and red cell distribution width–coefficient of variation (RDW-CV) were extracted from a complete blood count that was directly calculated using Sysmex SNL 550 (Syesmex, Hyogo, Japan) with impedance and photometric methods.

Erythrocyte SOD Activity Measurement

Erythrocyte SOD was calculated using Superoxide Dismutase Assay Kit (Catalogue No. 706002; Cayman Chemical, Ann Arbor, MI, USA) and Microplate Reader Biorad model 680 (Bio-rad Laboratories, Hercules, CA, USA) with software Microplate Manager ver 5.2.1 (Bio-rad Laboratories). Standard preparation for SOD calculation using 20 µL of standard SOD and dissolved with 1.98 mL of sample buffer to obtain a stock solution of standard SOD. Standard well SOD was made by adding 200 uL of the dissolved Radical Detector and 10 µL of standard was added per well to the plate. Whereas the sample was made by adding 200 µL of the dissolved Radical Detector and 10 µL of the sample was added into the well. Dissolved

xanthine oxidase was added immediately to all wells filled with standards and samples. The plate was incubated for 30 minutes on a shaker at room temperature. The absorbance was read at a wavelength of 450 nm using an enzyme linked immunosorbent assay (ELISA) plate reader. The results were calculated by calculating the average absorbance of each standard and sample. The linear rate of the standard was calculated based on the standard LR equation X = Standard Abs X/Standard Abs A. The next step was calculation of the linear rate of each sample based on the sample LR equation X = Abs sample X/Standard Abs A. After both linear rates were calculated, the linear rate of the standard was plot as a function of final SOD activity (U/mL).

Trace Elements Calculation

The examined panel of erythrocyte trace elements consisted of 15 variables, namely Vanadium (V), Cromium (Cr), Molybdenum (Mo), Mn, Fe, Cobalt (Co), Nickel (Ni), Cu, Zn, Se, Arsenic (As), Cadmium (Cd), Mercury (Hg), Thalium (Tl), and Lead (Pb). The calculation of trace elements was done using Agilent inductively coupled plasma mass spectrometry (ICP-MS) 7700x (Agilent, Santa Clara, CA, USA). Reference standard with ICP Multi-Element Standard Solution VI (Cat. 5185-5959, Merck, Rahway, NJ, USA), internal standards Indium Standard (Merck), reagent Ammonium solution 25%, Tritiplex III, Trition X, 1-Butanol, and Nitric acid 65% were used. One mL of erythrocyte sample was lysed with the addition of 1 mL of cold water, then the sample was diluted with a dilution factor of 100 times with an alkaline solution containing an internal standard of Indium for examination of metals V, Cr, Mn. Fe, Co, Ni, Cu, Zn, Se. Then, it was diluted 20 times with alkaline solution containing an internal standard of Indium for examination of metals As, Cd, Hg, Tl, Pb. Lastly, it was injected into the ICP-MS 7700x system with the MassHunter software (Agilent). The measurement of trace elements was carried out quantitatively and calculated against the standard using standard internal calculations. Erythrocyte trace elements were measured using concentration per erythrocyte. The examination was developed and validated by the Mass Spectrometry Laboratory of PT. Prodia Widyahusada for the research purpose only.

Results

Demographic Characteristics

The characteristics of the subject, including maternal age, maternal education, maternal occupation, blood pressure, gestational age, and body mass index (BMI) were presented in Table 1. There were differences in the level of education and blood preassure (both sistolic and diastolic) of preeclampsia subjects compared to normotensive subjects (p<0.05). For the BMI, most of preeclampsia subjects tend to be overweight or obese (65%), meanwhile in normotensive subjects most subjects had normal BMI (50%) followed by overweight or obese (45%).

Erythrocyte Index

Significant differences were found in the erythrocyte, MCV, MCH, and MCHC variables. The mean value of erythrocytes in preeclampsia subjects was higher than the normotensive subjects, with a mean difference of 0.55 million/mL (14.3%). As for the values of MCV, MCH, and MCHC, preeclampsia subjects had smaller values. This value indicated that the preeclampsia subjects had a smaller erythrocyte size than the normotensive subjects (Table 2).

Erythrocyte SOD Activity

Preeclampsia subjects had higher values of erythrocyte SOD activity than the normotensive subjects, both in terms of volume per mL of erythrocyte and per Hb. The difference in mean erythrocyte SOD/mL was 6.84 U/mL (23.6%), while SOD/Hb levels were 52.92 U/Hb (20.5%).

Erythrocyte Trace Elements Content

There were 15 variables of trace elements analyzed in this study, and Table 3 showed an example of the results of ICP-MS analysis results from one preeclampsia and one normotensive subjects. Cr was not detected in all study subjects. Five trace elements (V, Mo, Ni, Cd, and Tl) in some study subjects were not detectable. V was only detected in 6 preeclampsia subjects with a mean value of 0.27±0.55 g/g Hb. Meanwhile, in normotensive subjects, V was only detected in 1 subject with 0.15 g/g Hb levels. Mo was detected in 4 preeclampsia subjects with a median value of 1.75 g/g Hb. Among the normotensive subjects, Mo was only detected in 1 subjects with a value of 0.8 g/g Hb. The levels of Ni, Cd, and Tl were not detected in the preeclampsia and normotensive subject.

Based on the ICP-MS results, the levels of Fe, Co, Se, Cd, and Pb were significantly different in preeclampsia and normotensive subjects (Table 4). The levels of Fe, Se, and Cd were lower in the preeclampsia subjects than in the normotensive subjects, with differences of 10.6%, 14.7%, and 69.7%, respectively. The levels of Co and Pb were higher in the preeclampsia subjects, with a difference of 87.5% and 67.3%, respectively.

Table 1. Characteristics of subjects.

Variable	Preeclampsia (n=20)	Control (n=20)	*p*-value
Parity, n (%)			1.000
Nuliparity	7 (35)	7 (35)	
Multiparity	13 (65)	13 (65)	
Age (year), median (min-max)	32.8 (23-40)	32.0 (21-40)	0.547[b]
Gestational age (weeks), median (min-max)	30.8 (23-37)	30.8 (23-39)	0.947[b]
Education, n (%)			0.008[c,*]
College/University	19 (95)	12 (60)	
High school	1 (5)	8 (40)	
Occupation, n (%)			0.292[c]
Housewive	19 (95)	17 (85)	
Worker	1 (5)	3 (15)	
Sistolic (mmHg), mean±SD	168.1±19.2	114.2±12.9	0.000[a,*]
Diastolic (mmHg), median (min-max)	108 (80-130)	74 (67-96)	0.000[b,*]
Body Mass Index, n (%)			0.324[c]
Underweight (<18.5)	0 (0)	1 (5)	
Normal (18.5-25.0)	7 (35)	10 (50)	
Overweight/Obese (>25.0)	13 (65)	9 (45)	

[a]Tested with Independent T-test; [b]Tested with Mann-Whitney U test; [c]Tested with Chi Square test. *Significant if $p<0.05$.

Correlation of Erythrocyte SOD Activity and Other Variables

The correlation test of SOD activity on erythrocytes against erythrocyte trace elements was shown in Table 5. Only SOD levels with Fe levels had a significant negative correlation (r=-0.370). The lower Fe levels in erythrocyte were associated with increased SOD activity in erythrocyte. The correlation test of SOD activity compared to the patient's age or gestational age also gave a negative correlation with r=-0.218 and r=-0.259, but it was not statistically significant.

Discussion

The results of this study showed that erythrocyte SOD activity was higher in the preeclampsia subjects compared

Table 2. Distribution of complete peripheral blood in preeclampsia and control group.

Variable	Preeclampsia	Control	*p*-value
Hemoglobin (mg/dL)[a]	11.7±1.44	11.3±0.94	0.292
Hematocrite (%)[b]	35.8 (31.5-45.1)	33.7 (28.5-42.6)	0.072
Erythrocyte (million/mL)[a]	4.39±0.55	3.84±0.44	0.001*
MCV (fL)[a]	83.01±8.48	88.53±5.6	0.020*
MCH (pg)[a]	26.9±3.6	29.6±5.7	0.009*
MCHC (%)[a]	32.4±1.7	33.4±1.03	0.023*
RDW-CV[b]	14.3 (12.5-23.7)	14.1 (12-16.2)	0.448
Leukosit (/mL)[b]	13.65 (6.7-25.5)	11.8 (7.4-14.1)	0.056
Platelet (thousand/mL)[a]	281.8±122.9	258.4±56.8	0.446
ESR (mm/hour)[a]	52.35±27.1	52.65±22.2	0.97

[a]Normally distributed data presented in mean±SD, the difference in the mean was calculated by independent T-test. [b]Not normally distributed data presented in median (minimum-maximum value), the median difference is calculated by the Mann-Whitney U test. *Significant if $p<0.05$.

Table 3. An example of trace elements analysis using ICP-MS in normal and preeclampsia subject.

Normotensive Subject' Trace Elements									
RT	**Compound**	**Mass**	**Conc**	**Units**	**Count**	**Quant by**	**Det**	**Ratio**	**ISTD**
	Ni	60	1,043	ug/L	1.814	Area	Pulse	3,72E-02	In
	Cd	111	0,023	ug/L	53	Area	Pulse	1,10E-03	In
	Tl	205	0,145	ug/L	490	Area	Pulse	1,01E-02	In
	V	51	0,369	ug/L	759	Area	Pulse	2,93E-02	In
	Cr	52	<0.000	ug/L	4.292	Area	Pulse	1,66E-01	In
	Mn	55	0,709	ug/L	1.295	Area	Pulse	5,00E-02	In
	Fe	56	44,775	ug/dL	756.216	Area	Analog	2,96E+01	In
	Co	59	0,126	ug/L	344	Area	Pulse	1,33E-02	In
	Cu	63	1900,612	ug/L	2.962.639	Area	Analog	1,14E+02	In
	Zn	66	46,033	ug/dL	166.750	Area	Pulse	6,49E+00	In
	As	75	2,3	ug/L	1.011	Area	Pulse	3,91E-02	In
	Se	78	97,078	ug/L	4.832	Area	Pulse	1,87E-01	In
	Mo	95	0,622	ug/L	1.657	Area	Pulse		
	Hg	202	1,62	ug/L	860	Area	Pulse		
	Pb	208	0,008	ug/dL	866	Area	Pulse	3,35E-02	In
Preeclampsia Subject' Trace Elements									
RT	**Compound**	**Mass**	**Conc**	**Units**	**Count**	**Quant by**	**Det**	**Ratio**	**ISTD**
	Ni	60	1,252	ug/L	2.124	Area	Pulse	4,31E-02	In
	Cd	111	0,008	ug/L	46	Area	Pulse	9,26E-04	In
	Tl	205	0,152	ug/L	517	Area	Pulse	1,05E-02	In
	V	51	0,719	ug/L	1.427	Area	Pulse	5,41E-02	In
	Cr	52	<0.000	ug/L	5.112	Area	Pulse	1,94E-01	In
	Mn	55	0,815	ug/L	1.478	Area	Pulse	5,60E-02	In
	Fe	56	94,634	ug/dL	1.620.471	Area	Analog	6,14E+01	In
	Co	59	0,185	ug/L	488	Area	Pulse	1,85E-02	In
	Cu	63	2085,423	ug/L	3.312.311	Area	Analog	1,26E+02	In
	Zn	66	54,905	ug/dL	199.671	Area	Pulse	7,67E+00	In
	As	75	2,36	ug/L	1.057	Area	Pulse	4,01E-02	In
	Se	78	110,399	ug/L	5.566	Area	Pulse	2,11E-01	In
	Mo	95	0,95	ug/L	1.894	Area	Pulse		
	Hg	202	3,051	ug/L	1.569	Area	Pulse		
	Pb	208	0,012	ug/dL	1.057	Area	Pulse	4,00E-02	In

with normal pregnancy. This results was consistent with the finding of a study conducted in Spain that reported an increase in erythrocyte SOD activity in preeclampsia patients.(14) The increase may be due to an adaptive response from oxidative stress. Superoxide radicals can cause the body to convert non-radicals into radical products. Excessive radical oxygen species (ROS) can lead to many negative effects on female reproduction.(15–17) Thus, preeclampsia induces excessive radical oxygen species, which is immediately followed by the induction of endogenous antioxidant enzymes to strengthen the defense against oxidative injury. However, a study conducted in Turkey reported no difference in erythrocyte SOD activity in preeclamptic patients. In contrast, decreased erythrocyte SOD activity in preeclamptic women compared to controls was also reported.(6,18) The differences in this result could probably lie in the heterogeneity of this pathology.

Fe in erythrocytes can cause oxidative stress at low levels (iron deficiency anemia) and also at high levels (hemochromatosis) through the Fenton reaction.(19) The negative correlation of SOD activity with iron levels in erythrocytes can be explained by the fact that Fe is a contributor to free radicals in intracellular erythrocytes through the Fenton reaction. Transition metal ions such Fe and Cu are catalysts for the formation of oxygen radical species that react with hydrogen peroxide.(20,21) In this study, the Fe levels of erythrocytes were not examined, so the amount of free Fe was not known with certainty. However, some publications also mention a negative correlation between iron deficiency anemia with erythrocyte

Table 4. Distribution of erythrocyte SOD activity and erythrocyte trace elements.

Variable	Preeclampsia	Control	*p*-value
Erythrocyte SOD activity (U/mL)[a]	35.74±7.97	28.9±6.28	0.005*
Erythrocyte SOD activity/Hb (U/g Hb)[a]	310.8±83.4	257.88±63.1	0.029*
Mn (ag/erythrocyte)[b]	7.1 (4.3-13.7)	7.6 (4.7-12.4)	0.351
Fe (fg/erythrocyte)[b]	67 (23-82)	75 (24-92)	0.033*
Co (ag/erythrocyte)[b]	0.15 (0.05-0.61)	0.08(0.02-0.34)	0.027*
Ni (ag/erythrocyte)[b]	1.47 (0.08-4.28)	0.93 (0.12-3.39)	0.09
Cu (ag/erythrocyte)[b]	57.1(20.4-95.7)	58.5 (24.1-82.4)	1.000
Zn (fg/erythrocyte)[b]	1.4(0.5-2.0)	1.4(0.5-2.0)	0.850
As (ag/erythrocyte)[a]	0.46±0.19	0.39±0.09	0.091
Se (ag/erythrocyte)[a]	18.5±4.6	21.7±2.8	0.014*
Cd (ag/erythrocyte)[b]	0.10 (0.02-0.22)	0.33 (0.01-0.14)	0.006*
Hg (ag/erythrocyte)[b]	0.24(0.09-1.57)	0.29(0.09-1.38)	0.273
Tl (ag/erythrocyte)[b]	0.03 (0.02-0.1)	0.03 (0.02-0.06)	0.865
Pb (ag/erythrocyte)[a]	9.37±4.67	5.6±2.06	0.003*

[a]Normally distributed data presented in mean±SD, the difference in the mean was calculated by independent T-test. [b]Not normally distributed data presented in median (minimum-maximum value), the median difference is calculated by the Mann-Whitney U test. *Significant if $p<0.05$.

SOD activity.(22) Iron deficiency anemia could induce oxidative stress, which in turn responded by erythrocytes to increase antioxidant activity.(23,24)

In this study, it was found that the Se level of preeclampsia erythrocytes was lower than controls. Levels of Se in erythrocytes could have a protective effect on preeclampsia.(25) Se acts as a cofactor for the antioxidant glutathione peroxidase, which also fights free radicals. The antioxidant system of erythrocytes is not only SOD but several other enzymes were not included in this study. Decrease Se could be accompanied by antioxidant glutathione peroxidase in response to free radicals.(25)

Table 5. Correlation test of erythrocyte trace elements with erythrocyte SOD.

Trace Elements	Correlation coefficient (r)	*p*-value
Mn (ag/erythrocyte)[b]	-0.172	0.289
Fe (fg/erythrocyte)[b]	-0.370	0.019*
Co (ag/erythrocyte)[b]	0.059	0.717
Ni (ag/erythrocyte)[b]	0.247	0.152
Cu (ag/erythrocyte)[b]	0.023	0.886
Zn (fg/erythrocyte)[b]	-0.173	0.285
As (ag/erythrocyte)[a]	-0.124	0.445
Se (ag/erythrocyte)[a]	-0.115	0.481
Cd (ag/erythrocyte)[b]	0.163	0.407
Hg (ag/erythrocyte)[b]	-0.295	0.065
Tl (ag/erythrocyte)[b]	-0.011	0.957
Pb (ag/erythrocyte)[a]	-0.108	0.508

[a]Normally distributed data, tested using the Pearson correlation test. [b]Not normally distributed data, tested using the Spearman correlation test. *Significant if $p<0.05$.

There was a significant difference in the levels of Cd, Co, and Pb in erythrocytes compared to controls found in the current study. Although Cd is not the catalytic metal in the Fenton reaction, it induces oxidative stress in various animal models through indirect mechanisms.(21) The degree of Cd-induced oxidative stress depends on the dose, duration, and frequency of Cd exposure. Serum availability under experimental conditions, cell type, and antioxidant capacity, as well as Cd speciation, are important determinants.(26) At the cellular level, Cd-induced oxidative stress causes oxidative damage or activates signal transduction pathways to initiate a defensive response.(25) The role of Cd for cellular function in humans is still largely unknown. Still, Cd can induce oxidative stress response via metal transfer cellular redox status, decreased redox scavenger capabilities, inhibition of antioxidant enzymes, and inhibition of the electron transport chain resulting in mitochondrial damage. (27,28) A study in Boston on 1274 pregnant women with, 115 of them preeclampsia, it was found that Cd levels in erythrocytes were increased.(25) Population differences related to the environment, such as pollution and food, can influence this.

Co and Pb are toxic heavy metals that could be linked to preeclampsia. Erythrocytes' Co and Pb levels of preeclampsia in this study were found to be significantly higher than controls. However, previous research conducted in Boston found no differences in preeclamptic women's erythrocytes' Pb compared to controls.(25) However, in developing countries such as Congo, it was found that Co and Pb in urine were higher in preeclamptic women.(29)

Concentration of trace elements such as Cd, Hg, Pb, and Se was higher in erythrocytes than in plasma for maternal and umbilical cord blood samples.(30) Concentrations of Hg, Pb, and Se in maternal erythrocytes strongly correlated with their levels in the umbilical erythrocyte. In this study, there was no find significant differences in the levels of other trace elements: Ni, As, Hg, and Tl. Cr, V, and Mo levels were also not detected. Seeing the role of toxic metals such as Cd and Pb, N-Acetylcysteine therapy has the potential to be given to prevent preeclampsia. N-acetylcysteine is a derivative of cysteine, an amino acid, and can function as a chelator to remove metals, including Hg, Cd, Cr, As, and Pb.(31) However, N-Acetylcysteine does not appear to cause increased excretion of essential metals such as Fe, Zn, Cu, and Ca.(31)

In current study, only the status of antioxidants and trace elements were examined between both groups and did not directly describe the antioxidant stress that occurred. For further study, it would be better to examine NO levels in plasma or erythrocytes to help describe the maternal endothelial dysfunction in preeclampsia. Larger scale of research also needed to reduce the bias.

Conclusion

This study demonstrated significant differences in erythrocyte trace element levels between preeclampsia and normotensive patients. Specifically, Fe, Se, and Cd levels were lower in the preeclampsia group, while Co and Pb levels were higher. These findings suggest that alterations in erythrocyte trace element levels may serve as potential biomarkers for the prediction or management of preeclampsia.

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Authors Contribution

All authors were invoved in the study design and conception. SP and NW collected the data of the study. SP performed the analysis, interpretation of data and wrote the original draft of the manuscript. RI, YP, YBS, DP and NW revised the paper and had primary responsibility for the final content. All authors agreed to the published version of the manuscript.

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