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




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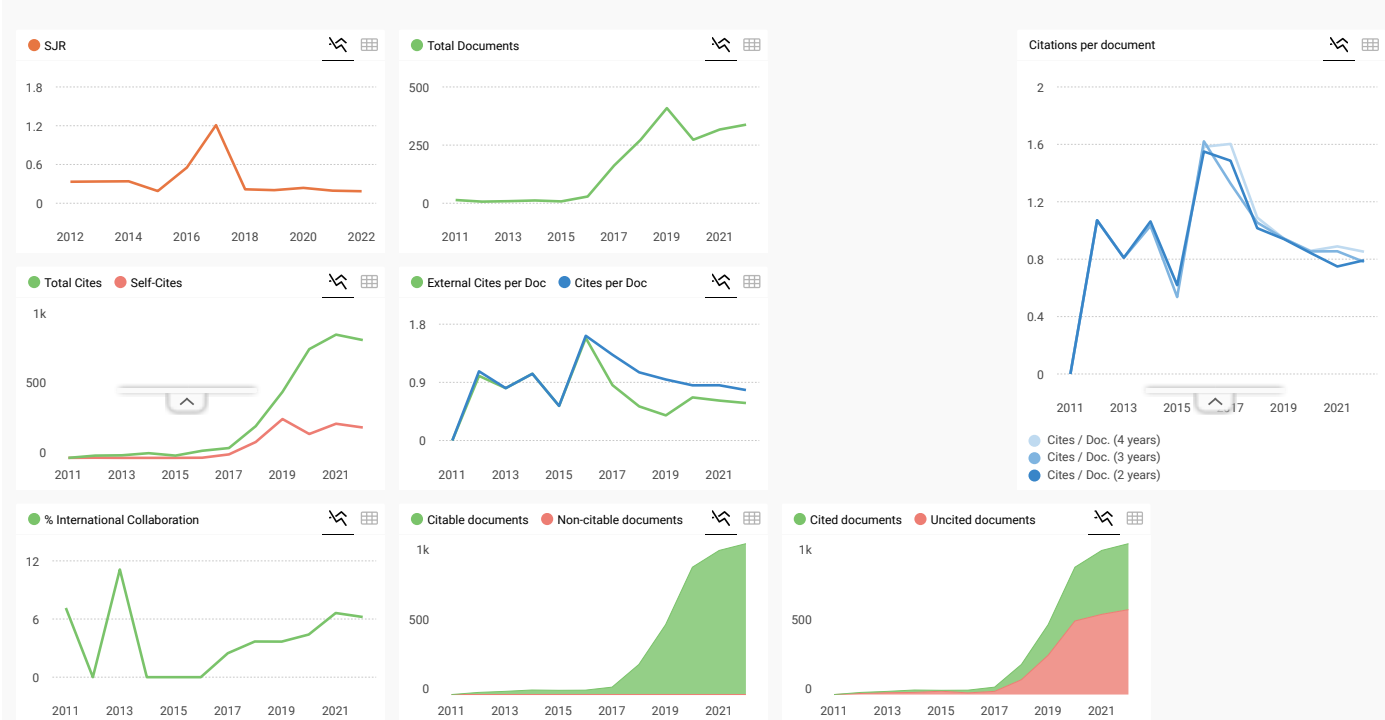
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## Vol 11, Special Issue 5 (Sep), 2019

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### EVALUATION OF CEFOPERAZONE/SULBACTAM AND VITAMIN K USE IN PATIENTS WITH BACTERIAL INFECTIONS

THEERAPONG SEESIN, PITIPONG PENGSAUPSIN, SARAWUT WEESAPHEN, PEERAYA SRIPHONG, UAEPONG LIMPAPANASIT, SIRIN BHONGCHIRAWATTANA

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Research Article

**STUDY OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE ENZYME AND ISOPROSTANE ON PREECLAMPSIA WITH NIFEDIPINE, METHYLDOPA, AND MAGNESIUM SULFATE THERAPY**

LINA LUKITASARI<sup>1\*</sup>, ADITIAWARMAN ADITIAWARMAN<sup>2</sup>, MASYHUDI MASYHUDI<sup>3</sup>, SITI KHAERUNNISA<sup>1</sup>, SUHARTATI SUHARTATI<sup>4</sup>

<sup>1</sup>Department of Medical Biochemistry, Faculty of Medicine, Airlangga University, Indonesia. <sup>2</sup>Department of Obstetrics and Gynecology, Faculty of Medicine, Airlangga University, Dr. Soetomo Hospital, Indonesia. <sup>3</sup>Department of Obstetrics and Gynecology, Muhamad Soewandi Hospital, Surabaya. <sup>4</sup>Department of Medical Biochemistry, Wijaya Kusuma Surabaya University, Indonesia. Email: lina-l@fk.unair.ac.id

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**ABSTRACT**

**Objective:** The objective of this research is to measure erythrocyte glucose-6-phosphate dehydrogenase (G6PD) enzyme activity and isoprostane and to correlate enzyme activity of G6PD with proteinuria and isoprostane in pregnant with proteinuria after the administration of nifedipine, methyl dopa, and magnesium sulfate.

**Methods:** This cross-sectional study was held in Soewandi Hospital, Surabaya, East Java, Indonesia. This study used total sampling as much as 30 pregnant women with proteinuria who got nifedipine, methyl dopa, and magnesium sulfate administration, age ranged from 17 to 48 years during their third trimester (>20 weeks). G6PD enzyme activity was measured from plasma by spectrophotometric method; plasma isoprostane was measured by competitive-ELISA method; and proteinuria urine spot was analyzed by urine dipstick from standardized laboratory of the hospital. Statistical analysis used in this study was Spearman's correlation coefficient.

**Results:** In this research, the effect of proteinuria +1 (OR=0.056) is lower than proteinuria +3 level on the presence of high G6PD enzyme activity, and proteinuria +2 (OR=0.933) is lower than proteinuria +3 level on the presence of high G6PD enzyme activity in pregnant women with proteinuria. G6PD enzyme was positively correlated (p=0.08) with proteinuria, and the connection was statistically significant. There was no significant statistic correlation between G6PD enzyme activity and isoprostane concentration (p=0.797).

**Conclusion:** This study found correlations between the enzyme activity of G6PD and proteinuria as the marker of renal damage in pre-eclampsia (PE) with the administration of nifedipine, methyl dopa, and magnesium sulfate. However, it had no correlation with isoprostane as the marker of oxidative stress. This study suggests that there should be a concern about understanding the pathophysiology of proteinuria for possibility of drug target for individuals with PE.

**Keywords:** Preeclampsia, Glucose-6-phosphate dehydrogenase enzyme, Proteinuria, Isoprostane, Nifedipine, Methyl dopa, Magnesium sulfate.

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**INTRODUCTION**

Preeclampsia is an emergency case in pregnancy due to pregnancy-induced hypertension (systolic pressure >140 mmHg, diastolic pressure >90 mmHg) which develops after 20 weeks of gestation complemented by one or more of the new proteinuria onset, maternal organ dysfunction and uteroplacental dysfunction [1]. Preeclampsia is a major cause of maternal and perinatal morbidity and mortality, around 3–10% of all maternal deaths in the world [2]. The incidence of preeclampsia (PE) has risen in low socioeconomic status, it might be associated with increased prevalence of chronic hypertension, obesity, and diabetes [3]. Indonesia as a developing country also has pre-disposition of PE. In Dr. Kariadi Hospital, Semarang, Central Java, Indonesia, PE incidence was around 2.45% and the main cause of maternal death around 40% in 1999 [4]. In Dr. Soetomo Hospital, Surabaya, East Java, Indonesia from July 2012 to June 2013, severe PE patients were about 461 patients and 24 patients (7%) developed into hemolytic elevated liver enzyme low platelet count syndrome complication [5].

Preeclampsia is a unique disease in pregnancy and requires crucial attention because the etiology and pathophysiology are still undetermined. A completely satisfactory and unifying hypothesis has not emerged. Schlembach (2003) calls this disease with the disease of theories, with one of the causes being oxidative stress [6]. Recent studies

have investigated the association between PE and G6PD deficiency. The result showed that there were incident of G6PD deficiency in PE and normal pregnancy although the correlation is not statistically significant [7,8]. However, the study also demonstrated that there was normal G6PD enzyme activity in PE. Research on G6PD deficiency in PE was rarely conducted overseas and none yet in Indonesia.

G6PD enzyme deficiency is an enzyme disorder with the highest prevalence, estimated at around 470 million of the entire world population in mid-2007 (6,625 billion) (7.09%). The diagnosis of G6PD enzyme deficiency occurs when G6PD enzyme activity was <60% which will cause clinical signs. Prevalence deficiency of G6PD enzymes in Indonesia in men is 5.9% and in women is 3.6%. In this study, there were 11 cases (35.5%) of 31 patients with G6PD deficiency and a history of poor pregnancy with no known cause [9-12].

Glucose-6-phosphate dehydrogenase (G6PD) enzyme has a role as endogenous antioxidant enzymes and a key of enzyme of pentose phosphate pathway that forms a part of glycolysis. G6PD is an enzyme for producing reduced nicotinamide adenine nucleotide phosphate and plays a role as coenzymes and acts by reducing glutathione and stabilizing catalase. G6PD deficiency causes increasing production of reactive oxygen species (ROS) leading to imbalance between oxidants and antioxidants called oxidative stress. Some study hypothesized that

there was association between the etiology of PE and stress oxidative by ROS [13-15].

Hypertension is tightly associated with progressive kidney dysfunction, with manifestation of glomerulosclerosis, interstitial fibrosis, proteinuria, and eventually declining glomerular filtration. It has been thought that tissue hypoxia induces fibrogenesis and progressive renal failure due to the generation of ROS. Renal tissue hypoxia decreases renal blood flow, due to high vascular tone induced by one of the mechanisms: oxidative stress yielding in increased mitochondrial oxygen, tubular electrolyte transport usage, and cutting off oxygen from arterial to venous blood in preglomerular vessels. Cross-sectional studies on the association between oxidative stress markers, isoprostane, and kidney function showed that isoprostane increase was commonly found in patients with established chronic kidney disease (CKD). Plasma F2-isoprostane, a marker of lipid peroxidation, was related to CKD stages and had been increased in patients with CKD compared to healthy controls [16,17]. Inhibition of angiotensin II (ANG II) activity, by inhibiting the activation of angiotensin-converting enzyme and blocking the receptor of ANG II type II are important to control hypertension and management with antioxidant will improve and protect the kidney tissues. Therefore, it seems important to find the possibility drug target for preeclamptic patients who have symptoms of kidney dysfunction [16,17]. Two ways for the management for PE are delivering the fetus (placenta) and pharmacological administration with medication of hypertension. In Indonesia, pharmacological administration protocol often used nifedipine 10-30 mg/day or methyldopa 0.5-3 g/day or intravenous administration of magnesium sulfate or combination. Methyldopa decreases norepinephrine (sympathetic outflow) to the heart, kidneys, and peripheral vasculature. Methyldopa has better result as antihypertension compared to labetalol [18]. Nifedipine is a calcium channel blocker mostly used in pregnancy because it can significantly drop blood pressure (BP) due to immediate release of oral nifedipine. Magnesium sulfate is not an antihypertensive drug but it has been used for seizure prevention in PE. The action of magnesium sulfate in preventing seizure is not completely unknown; however, it is thought to be due to the effect on the central nervous system, such as the n-methyl d-aspartate receptors, calcium channels, and acetylcholine [19].

## METHODS

This cross-sectional study was accomplished in the Muhamad Soewandi Hospital, Surabaya, East Java, Indonesia. Total sampling was conducted in pregnant women with hypertension and proteinuria. 30 preeclamptic women had come to the hospital with the inclusion criteria were >20-week gestational age, hypertension, and proteinuria. Pregnant women with a history of drug user, malaria, thalassemia, any thyroid diseases, and active smokers were excluded from this study. A total number of this study were 30 pregnant women of the third trimester (>20-week gestational aged) with hypertension and proteinuria. Enzyme activity of G6PD was measured by Spectrophotometric method using kit of Dialab at wavelength 450 nm. Proteinuria was measured by urine dipstick in a commercial clinical laboratory. Isoprostane was measured by competitive ELISA method using elabscience kit (Cat. No: E-EL-0041) at wavelength 450 nm. Data were expressed as mean  $\pm$  standard deviation. Spearman test was done as a test of significance wherever applicable. The statistical analysis was done using SPSS version 23. This study had been approved by medical and ethical committees of Medical Faculty of Universitas Airlangga, Surabaya, Indonesia, with certificate number 215/EC/KEPK/FKUA/2018.

## RESULTS AND DISCUSSION

Descriptive data are shown in Table 1. The results of the study obtained an average G6PD enzyme activity was 12.68 U/gHb (normal reference 4.8-12.5), proteinuria was 1.85 (negative normal reference), body mass index (BMI) was 31.07 kg/m<sup>2</sup> (reference overweight: 25-29.9; obesity Grade I: 30-34.9; obesity Grade II: 35-39.9; and obesity Grade III: >40), isoprostane level was 11.2 pg/mL, Hb level was 11.2 g/dL (normal reference 11.7-15.5), systolic BP was 160.43 mmHg (normal <140 mmHg), and diastolic BP was 93.6 mmHg (normal <90 mmHg). From these data, it could be indicated that PE patients who had been administered by nifedipine, methyldopa, and magnesium sulfate therapy had normal G6PD activity with values of proteinuria, BMI, systolic and diastolic BP were still above normal values based on reference.

Table 2 shows that there was significant relationship between G6PD enzyme activity variables with proteinuria, proteinuria with BP, and BP with the occurrence of PE in patients who had been treated with nifedipine, methyldopa, and magnesium sulfate. However, there was no significant relationship between isoprostane with G6PD or proteinuria. From the correlated data, the regression test was continued so that the results of causal relationships between variables could be found which can be seen in Table 3 and Fig. 1. The results showed that the relationship between G6PD enzyme activity and PE was hypothesized through variable proteinuria and BP (systolic and diastolic). G6PD activity correlates directly with systolic BP, systolic BP correlates directly with diastolic BP, and diastolic BP correlates directly with PE (Fig. 1).

The aim of this study was to correlate between G6PD enzyme activity, proteinuria, isoprostane, and BP in preeclamptic patients with nifedipine, methyldopa, and magnesium sulfate therapy. The result from this study showed that there was positive correlation between G6PD enzyme activity and proteinuria due to the production of ROS by hypertension (Table 2). Hypertension is strongly associated with increased oxidative stress and reduced PO<sub>2</sub> in the kidney, as a result, the risk for development of progressive kidney dysfunction will be increased. Furthermore, there is increasing evidence that oxidative stress contributes to and accelerates hypertension. Other studies indicate that BP induces ROS formation dependent on ANG II. ANG II induces arterial BP elevation and increases mitochondrial oxidative stress [17,20]. However, mitochondrial nitric oxide (NO) production increases as O<sub>2</sub> is reduced [13], which results in elevated levels of ONOO<sup>-</sup>, the end product reaction between O<sub>2</sub> and NO [21,22].

Some studies suggest that in PE patients, there is a large concentration of ROS in the placental and maternal circulation, and the antioxidant capacity is lower than the normal placenta, which causes oxidative stress. ROS production causes impaired remodeling, platelet aggregation, loss of vasodilation, inflammation and endothelial dysfunction [13]. One of the markers of oxidative stress is measuring the level of membrane lipid damage products, isoprostane [23]. Isoprostane is produced by the lipid peroxidation process. The result in this study was not showing correlation between G6PD enzyme activity with isoprostane [23]. The administration of the drug combination nifedipine, methyldopa, and



Fig. 1: Regression analysis between significant correlation variables

Table 1: Mean of G6PD, proteinuria, BMI, isoprostane, hemoglobin, systolic BP and diastolic BP of PE women with nifedipine, methyldopa, and magnesium sulfate

G6PD (U/g Hb)	Proteinuria (/UL)	BMI (kg/m <sup>2</sup> )	Isoprostane (pg/ml)	Hb (g/dL)	Systolic BP (mmHg)	Diastolic BP (mmHg)
12.68	1.85	31.07	651.62	11.2	160.43	93.6

G6PD: Glucose-6-phosphate dehydrogenase, BMI: Body mass index, BP: Blood pressure, PE: Pre-eclampsia

**Table 2: Statistical analysis; Spearman correlation between G6PD, proteinuria, isoprostane, and pre-eclampsia**

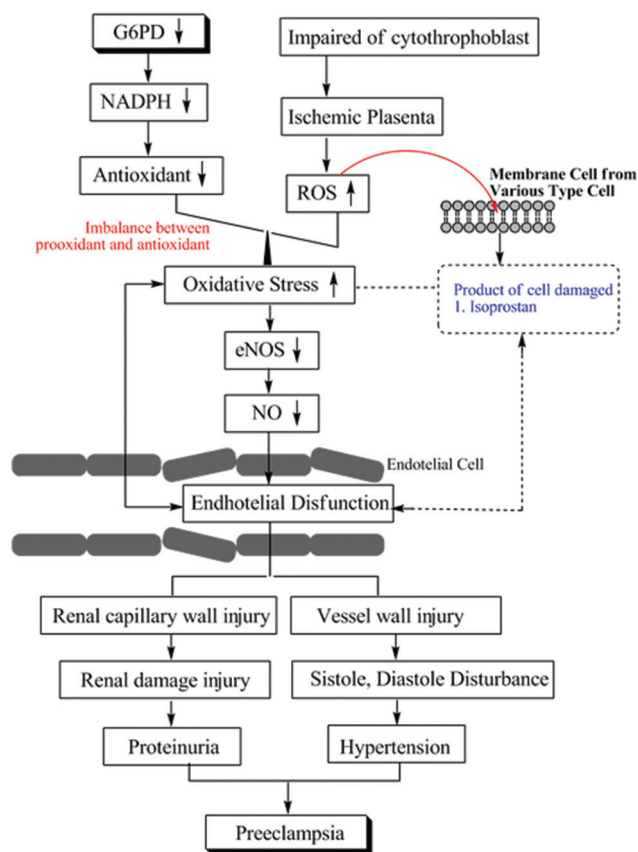
Variable 1	Variable 2	Spearman correlation (p)
G6PD	Proteinuria	p=0.008*
Proteinuria	Systole	p=0.021*
Proteinuria	Diastole	p=0.001*
Systole	Diastole	p=0.0001*
Proteinuria	BMI	p=0.036*
Systole	PE	p=0.017*
Isoprostane	G6PD	p=0.797
Isoprostane	Proteinuria	p=0.909

\*Significant. G6PD: Glucose-6-phosphate dehydrogenase, PE: Pre-eclampsia

**Table 3: Statistical analysis; regression analysis between significant correlation variables**

Variable 1	Variable 2	(p)	B
G6PD	Proteinuria	p=0.0001*	0.534
Proteinuria	Systole	p=0.019*	0.396
Systole	Diastole	p=0.0001*	0.665
Diastole	PE	p=0.037*	0.336

\*Significant. G6PD: Glucose-6-phosphate dehydrogenase, PE: Pre-eclampsia

**Fig. 2: Hypothesis of the association between glucose-6-phosphate dehydrogenase and endothelial dysfunction with the development of preeclampsia**

magnesium sulfate may be thought to have an effect on isoprostane level, which successfully controls the BP. High level of BP causes the excessive of ROS [16].

Based on the some study, we hypothesized (Fig. 2) that there were correlations between G6PD enzyme with proteinuria and isoprostane.

G6PD enzyme is an endogenous antioxidant enzymes. This enzyme function is reducing the ROS in the cells. If there is a disruption of the balance between oxidants and antioxidants, it can cause oxidative stress. ROS can enter the placental villi causing oxidative stress in placenta. Oxidative stress activates stress signaling (p38 $\alpha$  MAPK) and then activates the hypoxic transcription factor (1 $\alpha$ ) so that it will cause placental vascularization defects (failure in spiral artery remodeling) and cytotrophoblast only invades the maternal spiral arteries superficially. Vascular ischemia produces ROS. The concentration of ROS that accumulates from G6PD enzyme deficiency and placental ischemia will enter the systemic circulation, resulting in worsening oxidative stress in the cells. Vascular with systemic oxidative stress causes disturbance in nitric oxide synthetase (eNOS) mRNA and reduced endothelium eNOS transcription so that NO concentrations will be low. Low NO concentration results in endothelial dysfunction, therefore micro-macroangiopathy will be present in all cells. Renal microangiopathy causes impermeable glomerulus so that the protein can be detected in the urine. Damage of the walls of blood vessels results in high tense of blood vessel tone, increased systolic and diastolic BP (hypertension), so that proteinuria and hypertension occur in PE.

Based on the result and some study, it is necessary to give the patients pharmacological medicine in preeclamptic patients depends on the level of proteinuria and hypertension. Therapeutic target for preeclamptic patients focuses on the reduction of hypertension and the administration of antioxidant to prevent reduced kidney function.

## CONCLUSION

The present study found that there were correlations between the enzyme activity of G6PD and proteinuria as a marker of kidney damage in preeclamptic patients with nifedipine, methyldopa, and magnesium sulfate therapy. This study suggested that there was no association between G6PD enzyme activity and isoprostane as a marker of stress oxidative in preeclamptic patients with nifedipine, methyldopa, and magnesium sulfate therapy. However, there is still more possibility to do further studies on any drug target for PE by reducing risk factors of becoming eclampsia, reducing proteinuria and isoprostane. The present study suggests conducting further research by taking samples after the use of the drugs in PE.

## CONFLICTS OF INTEREST

All authors declared that there were not any conflicts of interest in this study.

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