R E S E A R C H   A R T I C L E

Transthyretin, Immature Platelet Fraction, and Mean Platelet Volume in Normotensive and Preeclampsia Pregnancy

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Abstract

BACKGROUND: Transthyretin protein experiences misfolding and aggregation in preeclampsia due to placental ischemia and inflammation. Placental endothelial damage occurring in preeclampsia stimulates the production of larger young platelets in the bone marrow and can be reflected by an increase in the immature platelet fraction (IPF) and mean platelet volume (MPV). Since the change in these markers in preeclampsia remains controversial, this study was conducted to analyze the differences in levels of transthyretin, IPF, and MPV as easily accessible markers in normotensive and preeclampsia pregnancies.

METHODS: Total subjects included in this study were 32 normotensive and 26 preeclampsia pregnant woman. The measurement of serum transthyretin level was using enzyme-linked immunosorbent assay (ELISA) method. Meanwhile, IPF and MPV levels were measured by using a hematology analyzer with flowcytometry method. Data was statistically analyzed using unpaired T-test and Mann-Whitney, with significancy of $p<0.05$.

RESULTS: Median value of transthyretin in normotensive and preeclampsia pregnancy were 11 (6-30) mg/dL and 9 (5-18) mg/dL. The mean value of IPF in normotensive and preeclampsia pregnancy were 4.56±2.19% and 6.33±2.99%. Mean value of MPV in normotensive and preeclampsia pregnancy were 10.41±0.76 fL and 11.32±1.2 fL. There were significant differences in levels of transthyretin, IPF, and MPV between normotensive and preeclampsia pregnancies ($p=0.008$, $p=0.017$, and $p=0.002$, respectively).

CONCLUSION: There are significant differences in transthyretin levels, IPF, and MPV values in normotensive and preeclampsia pregnancies. Therefore, serum transthyretin, IPF, and MPV can be developed as affordable parameters for the diagnosis of preeclampsia.

KEYWORDS: preeclampsia, transthyretin, IPF, MPV

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Introduction

Aside from bleeding and infection, preeclampsia is a worldwide health problem because it causes morbidity and mortality not only for the mother but also for the fetus.(1-4) The maternal mortality rate (MMR) in Indonesia is still one of the highest in Southeast Asia at 305/100,000 live births. Hypertension in pregnancy is the cause of 33% of maternal mortality in Indonesia and ranks first.(5)

Preeclampsia is considered a multisystemic disease with non-specific clinical features, but it usually characterized by increased blood pressure, however the exact cause is still unknown.(6-8) The existing theory shows the relation between preeclampsia and abnormal placentation, that is the failure of cytotrophoblast invasion to the spiral arteries. This condition causes placental ischemia and hypoxia, increases oxidative stress, endothelin metabolism disorder or endothelial dysfunction, and stimulates systemic inflammation.(9-11)

Transthyretin, a serum protein that transports thyroid hormones and retinol-binding protein, is prone to misfolding and aggregation due to endoplasmic reticulum stress and systemic inflammation in patients with preeclampsia. Previous studies have shown transthyretin as a key protein that experiences misfolding, aggregating, and dysregulating in preeclamptic placental tissue, playing a role in the pathogenesis of preeclampsia. It is necessery to assess changes in transthyretin levels in the serum of preeclamptic patients, since it is less invasive and less risky than placental tissue examination.(9,12-14)

Ischemia and oxidative stress of syncytiotrophoblasts that occur in preeclampsia make the placenta release proinflammatory cytokines and antiangiogenic factors that are responsible for endothelial dysfunction. An imbalance of angiogenic factors with anti-angiogenic factors will interfere with trophoblast invasion and the process of spiral artery changes, which play a role in the process of preeclampsia. (15-17) Endothelial dysfunction will activate platelets and increase aggregation, increase consumption of platelets in the peripheral and stimulate the process of thrombopoiesis in the bone marrow so that the bone marrow produces more young, large platelets. Immature platelet fraction (IPF) is a platelet with a higher amount of RNA that has just been released from the bone marrow. The amount of IPF will increase with increasing thrombopoiesis activity. The mean platelet volume (MPV) as a measure of the average size of platelets in circulation will also increase with an increase in the number of large, more metabolically active platelets, and a higher level of aggregation. However, changes in MPV in preeclampsia in existing studies are controversial. (18-20) This study was conducted to analyze the differences in levels of transthyretin, IPF, and MPV as easily accessible markers in normotensive and preeclampsia pregnancies.

Methods

Study Design and Sample Recruitment

This was an analytic observational study with a cross-sectional approach. Sampling was carried out from January to August 2021. Subjects of normotensive pregnancies were recruited in the Ngesrep Public Health Center Semarang, while subjects of preeclampsia were recruited in Dr. Kariadi Semarang Central General Hospital.

The inclusion criteria for this study were pregnant women aged 20-40 years old, pregnancy period >20 weeks, normal body temperature (36.5-37.5ºC), systolic blood pressure (SBP) <140 mmHg, and diastolic blood pressure

(DBB) <90 mmHg for normotensive pregnant women, and SBP≥140 mmHg or DBP≥90 mmHg with organ dysfunction criteria according to preeclampsia criteria for preeclamptic pregnant women. Subjects with a history of liver disease, diabetes mellitus, history of taking oral anticoagulants in the last 3 days, and smokers were excluded from this study. All research subjects were asked for their consent by signing a written informed consent before the study. The protocol of this study has been approved by the Health Research Ethics Commission of Dr. Kariadi Semarang Central General Hospital (No. 750/EC/KEPK-RSDK/2021).

Data Collection

Data collection included anamnesis, physical examination, interview, and blood collection. Blood was taken from the median cubital vein using a vacutainer of 8 cc: 2 cc for the tube without anticoagulant, and 3 cc each to be inserted into 2 ethylenediaminetetraacetic acid (EDTA) tubes. Blood without anticoagulant was centrifuged to obtain serum and transthyretin levels were measured. EDTA blood was examined with a hematology analyzer to obtain IPF and MPV values.

Transthyretin, IPF, and MPV Examination

The measurement of transthyretin levels was conducted using enzyme-linked immunosorbent assay (ELISA) sandwich method Elx800 (Biotek, Vermont, NE, USA) with reagent from Elabscience (Houston, TX, USA). The micro ELISA plate was pre-coated with an antibody specific to human transthyretin. Standards and samples were added to the micro ELISA plate wells and combined with the specific antibody. A biotinylated detection antibody specific for human transthyretin protein and avidin-horseradish peroxidase (HRP) conjugate were added to each micro plate well and incubated. The substrate solution was added to each well after washing. The optical density (OD) was measured spectrophotometrically at 450 nm and the OD value was proportional to the concentration of human transthyretin.

A complete hematology examination to obtain MPV values was carried using hematology analyzer Sysmex XN 1000 (Sysmex, Kobe, Japan) with impedance method. The cells in suspension passed through a small aperture between the two chambers where an electric current available. As each cell passed, it created an impulse, changed the resistance of the electrical current that was proportional to the volume of the cell crossing the aperture.

Meanwhile, the IPF examination was performed using hematology analyzer Sysmex XN 2000 (Sysmex) which adopted fluorescent flow cytometry with a semiconductor

diode laser. The platelet membrane was initially perforated by the reagent. Furthermore, oxazine fluorescent dyes penetrated the platelets and specifically labeled the RNA inside the platelets. The stained platelets were passed through a semiconductor diode laser beam. IPF was expressed as a proportional value (%-IPF) of the total platelet count.

Statistical Analysis

Statistical analysis was performed using the IBM Statistical Package for the Social Sciences (SPSS) Statistics 23 program (IBM Corporation, Armonk, NY, USA). Each variable was tested for normality using the Saphiro-Wilk test. The MPV data were normally distributed, and the IPF data which were normally distributed after the transformation was tested using an unpaired T-test. Transthyretin data that were not normally distributed after transformation were analyzed by the Mann-Whitney test. The parity and trimester characteristics of the sample were tested by chi-square, and other sample characteristic data were tested by unpaired T-test or Mann Whitney. The data has significance if the $p<0.05$ with a 95% confidence interval range value.

Results

The mean age of normotensive subjects (28.94 ± 6.15 years old) was lower than the preeclampsia subjects (29.73 ± 5.67 years old). The gestational age of normotensive and preeclampsia subjects was dominated by the third trimester, 90.6% and 92.3%, respectively. The parity observed in this study was dominated by multiparity, 65.6% in normotensive subjects and 69.2% in preeclampsia subjects, with the predominance of a second pregnancy (53.1% and 38.4%, respectively). The characteristics data of the research subjects were shown in the Table 1.

Table 1. Subjects' characteristics data.

Variable	Normotensive (n= 32)	Preeclampsia (n= 26)	p- value
Gestation, n (%)			
1	11 (34.4%)	8 (30.8%)	
2	17 (53.1%)	10 (38.5%)	0.25^{χ}
3	4 (12.5%)	6 (23.1%)	
4	-	2 (7.7%)	
Trimester, n (%)			
2	3 (9.4%)	2 (7.7%)	0.82^{χ}
3	29 (90.6%)	24 (92.3%)	
Gestational age (week), median (min-max)	33.5 (22-39)	36 (25-40)	0.378^{M}
Age (tahun), mean±SD	28.94±6.15	29.73±5.67	$0.615^{\dagger}$
SBP (mmHg), mean±SD	115.53±8.79	153.04±10.74	$0.000^{*\dagger}$
DBP (mmHg), median (min-max)	78 (60-89)	94.5 (85-118)	0.000^{*M}
Heart Rate (/min), median (min-max)	86 (64-96)	89 (78-111)	0.027^{*M}
Respiratory Rate (/min), median (min-max)	20 (18-22)	20 (18-26)	0.764^{M}
Hemoglobin (g/dL), mean±SD	11.21±1.29	11.76±1.69	$0.165^{\dagger}$
Hematocrit (%), mean±SD	33.65± 3.24	35.38±4.29	$0.085^{\dagger}$
Erythrocyte (x106/uL), mean±SD	3.98±0.35	4.18±0.57	$0.103^{\dagger}$
RCDW (%), median (min-max)	14.2 (12.3-19.9)	14.85 (12.8-27.5)	0.110^{M}
Leukocyte ($x10^{3}$/uL), median (min-max)	8.7 (3.6-13.9)	9.1 (3.6-13.4)	0.309^{M}
Thrombocyte ($x10^{3}$/uL), mean±SD	263.56±70.97	244.08±59.92	$0.27^{\dagger}$

SBP: systolic blood pressure; DBP:diastolic blood pressure; RCDW: red cell distibution width. [M]Tested with Mann Whitney; [†]Tested with unpaired T-test; [χ]Tested with Chi square; *statistically significant ($p<0.05$).

Analysis of differences in transthyretin levels in normotensive and preeclampsia pregnant subjects was done using the Mann-Whitney test, and significant difference was obtained (p=0.008). Meanwhile the analysis of differences in IPF and MPV was performed by unpaired T-test. Both IPF and MPV value in normotensive and preeclampsia subjects were significantly different (p=0.017 and p=000.2, respectively (Table 2).

Discussion

The mean age of normotensive pregnancies in this study was 28.94±6.15 years, while the peeclampsia subjects was 29.73±5.67, which was higher than normotensive pregnancies. Advancing age during pregnancy increases the risk associated with aging of the uterine vessels and arterial stiffness, leading to a gradual loss of vascular compliance, leading to endothelial dysfunction, and subsequently leading to higher afterload.[21]

The parity observed in this study was dominated by multiparity, 65.6% in normotensive group and 69.2% in preeclampsia group, with the predominance of second pregnancy. There was no significant difference in parity between the two groups in this study, as well as the trimester in the two groups. The increased risk of multiparous preeclampsia can occur at intervals between births less than 5 years, changes in partners, history of low weight births and previous premature births.[22] Data regarding risk factors for preeclampsia in multiparous have not been assessed in this study.

The measurement of the platelet count of the two groups in this study showed no significant difference, but the mean value of normotensive platelets (263.56±70.97 x10³/uL) was higher than the number of preeclampsia platelets (244.08±59.92 x10³/uL). These results are in line with previous studies that did not find a significant decrease in platelets in preeclampsia patients. An insignificant decrease in platelets despite an increase in peripheral platelet consumption may be possible due to an increase in bone marrow platelet production, as evidenced by an increase in IPF values.[20,23]

The median serum transthyretin levels of normotensive pregnancies (11 (6-30) mg/dL) in this study showed higher values than preeclampsia patients (9 (5-18) mg/dL) with a statistically significant difference (p=0.008). This is in line with previous studies, which showed the median serum transthyretin in severe preeclampsia patients was significantly lower than the median transthyretin of normotensive pregnancies.[24] The transthyretin levels in the normotensive and preeclampsia pregnant groups in this study were lower than the reference values in normal adults, this is presumably due to dilution as well as an increase in urinary protein excretion during pregnancy. Serum transthyretin began to decrease progressively since 12 weeks of gestation, where the sample of this study was dominated by third trimester pregnant women.[25,26]

Previous studies showed a characteristic more intense immunohistochemical coloration for transthyretin in fibrotic stromal material in villi and areas of a preeclamptic placental infarct. The study also found a significant increase in monomer transthyretin in the amniotic fluid of preeclampsia patients, indicating a change in transthyretin in the process of preeclampsia.[27,28]

Oxidative stress due to placental ischemia in preeclampsia can affect the stability of the functional form of transthyretin dimers which stimulates the formation of monomeric proteins more quickly and tends to form aggregations.[27,29] Exaggerated inflammatory response in preeclampsia also has a positive effect on transthyretin amyloid formation by inducing transthyretin fibril aggregation and decreasing transthyretin serum level.[30]

The mean IPF value of normotensive pregnant (4.56±2.19%) in this study was lower than the IPF of preeclamptic subjects (6.33±2.99%), with a statistically significant difference of p=0.017. The results of this study

Table 2. Differences in transthyretin, IPF, and MPV levels in normotensive and preeclampsia pregnant subjects.

Variable	Normotensive (n= 32)	Preeclampsia (n= 26)	*p*-value
Transthyretin (mg/dL), median (min-max)	11 (6-30)	9 (5-18)	0.008*[M]
IPF (%), mean±SD	4.56 ±2.19	6.33±2.99	0.017*[†]
MPV (fL), mean±SD	10.41 ±0.76	11.32±1.2	0.002*[†]

IPF: immature platelet fraction; MPV: mean platelet volume; [M]Tested with Mann Whitney; [†]Tested with unpaired T-test; *statistically significant (p<0.05).

are in line with other research.(20) Decreased amount of platelet occurs in preeclampsia patients associated with hemodilution, and an increase in platelet consumption followed by an increase in platelet turnover, causing expansion and differentiation over several days, followed by rapid reorganization. The new immature platelets are taken out more significantly and more reactive than an adult platelets, containing RNA, producing IPF scores on the blood test.(31,32)

In line with the increase in IPF, the increase in MPV value reflects an increase in young platelets of larger size, indicating an active turnover of platelet production in the bone marrow due to peripheral consumption.(23,33) The mean MPV value in this study showed that the normotensive pregnant women group (10.41±0.76 fL) was lower than the preeclampsia group (11.32±1.2 fL) with a significant difference (p=0.002).

Previous studies carried out MPV measurements at two times, namely before 20 weeks of gestation and just before caesarean section, and found higher MPV values in preeclampsia than in normotensive pregnancies, indicated that systematic backup and platelets activation are already available since the early pregnancy, and MPV can work as an additional predictor of preeclampsia.(34) An increase in MPV is a sensitive indicator of platelet activation and consumption, and these parameters are routinely reported on complete blood count which is commonly performed in clinical laboratories.(33)

The results of studies examining the relationship between preeclampsia and MPV are still contradictory. The different results in these studies could be related to the use of the analytical method, the use of the type of anticoagulant, and the time required from specimen collection to specimen analysis. Other factors such as study design and numbers of samples may also have contributed to these differences.(19,35,36) This study did not classify the degree of preeclampsia, which may show clearer changes in transthyretin, IPF, and MPV in hypertension in pregnancy patients. Further research is needed regarding the use of these parameters as markers of preeclampsia.

Conclusion

There are significant differences in transthyretin levels, IPF, and MPV values in normotensive and preeclampsia pregnancies. Therefore, serum transthyretin, IPF, and MPV can be developed as affordable parameters to help establish the diagnosis of preeclampsia.

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