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Differences in maternal soluble ST2 levels in the third trimester of normal pregnancy versus preeclampsia

Prameswari Hawani Sasmaya[a,*], Khalid Achmad Fitrah[a], Anggraeni Dewi[a], Irianti Setyorini[b], Akbar Mohammad Rizki[a]

[a] Department of Cardiology, Hasan Sadikin General Hospital, Universitas Padjadjaran, Bandung, West Java, Indonesia
[b] Department of Obsetrics and Gynecology, Hasan Sadikin General Hospital, Universitas Padjadjaran, Bandung, West Java, Indonesia

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ABSTRACT

Background: Preeclampsia is associated with intense inflammatory response in pregnancy, and soluble ST2 (sST2) is pathologically increased in this condition. No data exist regarding maternal sST2 levels in normal pregnancy versus preeclampsia in areas of southeast Asia with an ethnic Malay predominance.
Materials and Methods: Patients were sorted into normal pregnancy or preeclampsia. Patients with a history of allergic, inflammatory, or malignant disease were excluded. One sample was taken per patient; all samples were taken during the third trimester of pregnancy. Thirty samples from each group were enrolled in the study, totaling 60 samples. Soluble ST2 levels in maternal plasma were measured using the Presage® ST2 Assay according to manufacturer instructions, and data was analyzed using SPSS 23.
Results: Patients in the preeclampsia group were significantly older than those in the normal pregnancy group ($p = 0.01$). Most patients with preeclampsia presented as early-onset ($n = 23$). Both systolic blood pressure (SBP) and diastolic blood pressure (DBP) were significantly higher ($p < 0.001$) in the preeclampsia group. Mean sST2 level in the preeclampsia group (85.89 ng/ml) was significantly higher than the normal pregnancy group mean (38.3 ng/ml) during the third trimester ($p < 0.001$). This study also found a correlation between sST2 and preeclampsia ($p < 0.001$, $r = 0.480$), SBP ($p < 0.001$, $r = 0.407$), and DBP ($p = 0.007$, $r = 0.342$), while preeclampsia was found to be the best explanatory variable of sST2 levels ($r = 0.468$, $p < 0.001$). sST2 level > 63.66 ng/ml has sensitivity 50% and specificity 93.3%, with AUC of 0.78 [95% CI 0.66 – 0.90], $p < 0.001$. The sST2 > 63.66 ng/ml has an OR of 14.0 [95% CI 2.82 – 69.6], $p < 0.001$ for preeclampsia. The dose-response relationship between sST2 level and preeclampsia was linear.
Conclusion: Soluble ST2 levels were increased in both normal pregnancy and preeclampsia but were significantly higher in patients with preeclampsia. Preeclampsia was also found to be the best explanatory variable for the increase of sST2 levels in ethnic Malay predominance.

Introduction

Novel high-sensitivity soluble Suppression of Tumorigenicity-2 (sST2) assays have been developed and used as valuable adjuncts for prognosis and monitoring of Heart Failure (HF) and were included in the 2017 American College of Cardiology/American Heart Association update of heart failure guidelines [1]. Soluble ST2 was found to play an important role in cardiovascular disease, not only as a predictor of hospitalization and death but also as a factor in determining the prognosis of heart failure patients [2,3].

Suppression of Tumorigenicity-2 (ST2) is a member of the IL-1 family of proteins. Due to alternative splicing, ST2 is found in multiple forms, including a transmembrane form (ST2L) and a soluble circulating form.[4]. ST2, together with its ligand IL-33, is associated with a Th2 immune response via the production of anti-inflammatory cytokines [5,6]. In HF, the higher levels of sST2 are thereby associated with increased myocardial fibrosis, adverse cardiac remodeling, and worse cardiovascular outcomes [7–9].

Preeclampsia is characterized by an intense inflammatory response associated with a shift to dominance of pro-inflammatory cytokines associated with T helper 1 cells. However, normal pregnancy also displays a mild systemic inflammatory response and an

* Correspondence to: Eyckman no.38, Bandung 40161, West Java, Indonesia.
E-mail addresses: hawanisasmaya@gmail.com (P.H. Sasmaya), achmdfitrah@gmail.com (A.F. Khalid), anggraenidewidr@gmail.com (D. Anggraeni), driranti0901@gmail.com (S. Irianti), m_rizki_a@ymail.com (M.R. Akbar).

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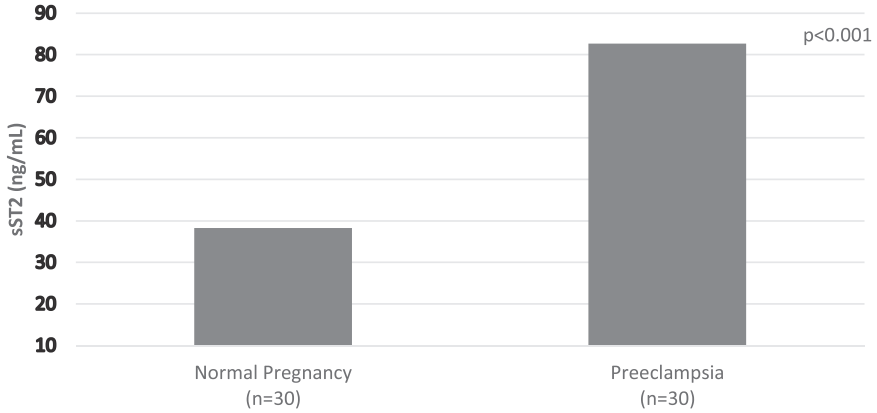

Fig. 1. Comparison of sST2 Levels in Normal Pregnancy vs. Preeclampsia (mean = 38.3 ng/ml and 82.7 ng/ml, respectively; $p < 0.001$).

immune bias towards type 2 cytokine production. Previous research has demonstrated a significant increase in sST2 levels in both normal pregnancies and preeclamptic pregnancies, particularly in the third trimester versus the first and second trimesters. Soluble ST2 levels have also been found to be significantly higher in the third trimester of preeclamptic pregnancies versus the third trimester of normal pregnancies. Endothelial dysfunction, placental secretion, ventricular hypertrophy, and remodeling have all been linked with elevated levels of sST2 in patients with preeclampsia [10–12]. Despite these findings, no data regarding maternal sST2 levels in normal pregnancy and preeclampsia in southeast Asian populations exist. Addressing this lack of data is vital, as these populations represent a distinctly different ethnic group, with different background clinical characteristics, compared to Western populations. To the best of our knowledge, this is the first study comparing and analyzing the levels of sST2 in normal versus preeclamptic pregnancies in areas of southeast Asia with an ethnic Malay predominance.

Materials and methods

Subject recruitment and ethics statement

This study was approved by the West Java Research Ethics Committee, and written consent was obtained from each participant. Patients considered eligible for this study met the following criteria: the patient (1) must be within the third trimester of pregnancy (> 30 weeks); (2) must have a history of at least one visit to the designated hospital (Dr. Hasan Sadikin General Hospital and Bandung's General Hospital); (3) must be diagnosed with preeclampsia or normal pregnancy by a certified obstetrician; and (4) must have no history or signs of allergic, inflammatory, or malignant disease. Preeclampsia was defined as the new onset of systolic blood pressure (SBP) > 140 mmHg or diastolic blood pressure (DBP) > 90 mmHg after the 20th week of gestation accompanied by new-onset proteinuria. Proteinuria was defined as > 300 mg of protein in a 24-hour urine collection period, a protein/creatinine ratio of 50 mg/mmol, or a reading of at least 2 + in dipstick testing for two consecutive measurements.

To compare sST2 levels in the third trimesters of patients with normal pregnancies versus those with preeclampsia, two groups were formed. A single blood sample was taken from those meeting the inclusion criteria; plasma samples were collected following centrifugation of blood samples in EDTA anticoagulant tubes. Given that cardiovascular risk complications commonly occur during the third trimester of pregnancy, each patient's sample was collected during the first clinical visit in their third trimester.

Sample collection and detection of sST2 by ELISA

Blood samples were collected in tubes containing EDTA. Samples were centrifuged for 15 min at 4 °C and stored at 2–8 °C. Levels of sST2 in maternal plasma were measured using the Presage® ST2 Assay according to manufacturer instructions.

Statistical analysis

The Kolmogorov-Smirnov test was used to assess the distribution of the data. Normally distributed data were analyzed using Pearson's test, while non-normally distributed data were analyzed using the Mann-Whitney test to compare variables (e.g., age, BMI, blood pressure, gestational status) between the groups. All probability values were deemed statistically significant at a level of < 0.05, and for variables with $p < 0.25$ during bivariate analysis, multivariate analysis was then used to determine the best explanatory variable for the difference in sST2 levels in preeclampsia versus normal pregnancy during the third trimester. We performed a receiver operator curve (ROC) analysis to generate area under the curve (AUC), sensitivity, and specificity at a specific cut-off point. We constructed a restricted cubic spline to evaluate the non-linearity of dose-response relation between sST2 and preeclampsia. Statistical analysis was performed using SPSS 23.

Results

This study included a total of 60 samples; 30 samples were obtained from normal pregnancies and 30 were obtained from preeclamptic pregnancies. All samples were taken during the third trimester of pregnancy. Patients who developed preeclampsia were significantly older than patients with normal pregnancies ($p = 0.01$). There was no significant difference in body mass index between the two groups. Gravida, parity, and miscarriage history were significantly higher in the preeclampsia group versus the normal pregnancy group. Preeclampsia was mostly diagnosed at less than 34 weeks of pregnancy, which was considered an early onset of preeclampsia (n = 23). Most of the normal pregnancy group underwent vaginal delivery method, while most of the preeclampsia group underwent cesarean section. Both systolic and diastolic blood pressure were significantly higher in the preeclampsia group versus the normal pregnancy group ($p < 0.001$). Soluble ST2 levels in the preeclampsia group were also significantly higher than those in the normal pregnancy group ($p < 0.001$; Fig. 1). The demographic and clinical characteristics of the study groups are displayed in Table 1.

There was no significant correlation between sST2 levels and age, BMI, and gestational status. However, a significant positive correlation was found in preeclampsia or normal pregnancy at enrollment

Table 1
Clinical characteristics of women with normal pregnancies vs. preeclampsia (n = 60).

	Normal pregnancy group (n = 30)	Preeclampsia group (n = 30)	P value
Age	28 (4.8)	33.1 (6.2)	0.01
Body Mass Index (kg/m^2)	23.5 (2.3)	25.1 (4.1)	0.10
Blood pressure	111.6 (8.7)	163.6 (14.7)	<0.001
SBP	73.3 (6)	102 (12.4)	<0.001
DBP			
Gestational status (at enrollment)	32.4 (2.6)	33.13 (2.8)	0.24
Gestational Age (weeks)	2 (0.9)	2.7 (1.1)	0.01
Gravida	0.9 (0.9)	1.4 (0.9)	0.04
Parity	0.03 (0.1)	0.2 (0.5)	0.04
Miscarriage			
Mode of delivery	27 (90%)	5 (16%)	
Vaginal delivery (%)	3 (10%)	25 (84%)	
Cesarean section (%)			

Data are shown as mean (+/- standard deviation); SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure

Table 2
Correlation between sST2 levels and variables.

	P Value
Age	0.25
Preeclampsia/normal pregnancy	<0.001
Body Mass Index (kg/m^2)	0.64
Gestational status (at enrollment)	0.82
Gestational Age (weeks)	0.60
Gravida	0.51
Parity	0.83
Miscarriage	
Blood pressure	<0.001
SBP	0.007
DBP	

SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure

Table 3
Multivariate analysis between sST2 levels and variables.

Steps	Variables	Coefficient	Coefficient correlation	P value
Step 1	PE/Normal Pregnancy	58.311	.615	.039
	Age	-0.486	-0.062	.638
	SBP	.021	.013	.970
	DBP	-0.435	-0.158	.526
	Constant	23.254		.584
Step 2	PE/Normal Pregnancy	58.995	.622	.006
	Age	-0.481	-0.062	.636
	DBP	-0.422	-0.154	.469
	Constant	23.798		.548
Step 3	PE/Normal Pregnancy	56.292	.593	.006
	DBP	-0.414	-0.151	.475
	Constant	12.411		.691
Step 4	PE/Normal Pregnancy	44.426	.468	.000
	Constant	-6.076		.728

PE: Preeclampsia; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure

(r = 0.480) and blood pressure, both systolic and diastolic blood pressure (r = 0.407 and r = 0.342, respectively). Based on multivariate analysis, preeclampsia or normal pregnancy at enrollment was determined to be the best explanatory variable for the elevation of sST2 levels in this study (r = 0.468), independent of age, BMI, gestational status, and blood pressure. All analysis between variables and sST2 levels is listed in Table 2 and Table 3. Soluble ST2 level > 63.66 ng/ml has sensitivity 50% and specificity 93.3%, with AUC of 0.78 [95% CI 0.66 – 0.90], p < 0.001 [Fig. 2]. The sST2 > 63.66 ng/ml has an OR of 14.0 [95% CI 2.82 – 69.6], p < 0.001 for preeclampsia. The dose-response relationship between sST2 level and preeclampsia was linear [Fig. 3].

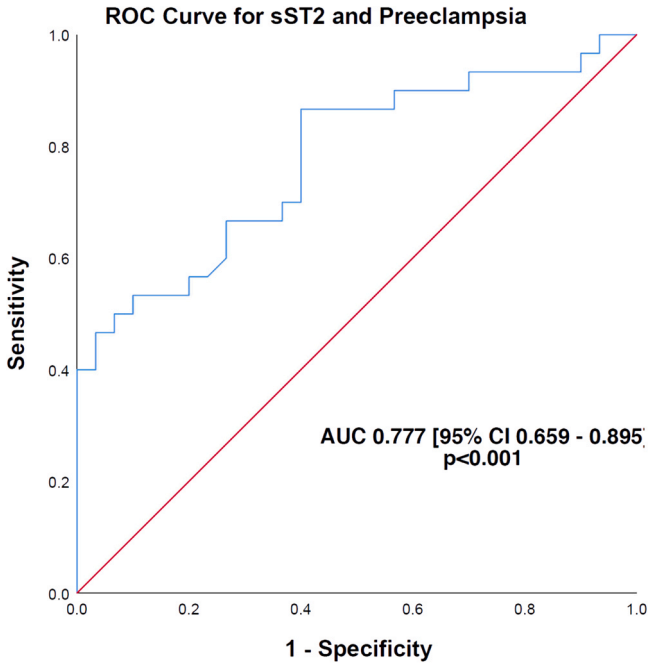

Fig. 2. ROC curve for sST2 and preeclampsia.

Discussion

This study results showed that sST2 levels were significantly increased in the third trimesters of women with normal pregnancies (mean = 38.3 ng/ml) and further increased in those with preeclampsia (mean = 85.89 ng/ml). Multivariate analysis also found normal pregnancy and preeclampsia to be the best explanatory variable for sST2 levels. Previous studies have demonstrated a pathological increase of sST2 levels in patients with preeclampsia [10,13,14]. Our results, which were obtained primarily from patients of Malay ethnicity, exhibited similar sST2 characteristics in normal pregnancy and preeclampsia to those of Western populations, which are composed of different ethnic groups than those considered in the current study. Although there was a tendency of ethnicity impact to the level of sST2, in our study we found a similar pattern with the previous study conducted by Granne et al. However, results from this study displayed higher sST2 levels in normal pregnancy compared to the previous study [10]. Regardless of the role ethnicity plays, since sST2 levels were not significantly influenced by age and BMI, our differences may have been caused by the range of gestational ages during blood collection. Our study had a slightly wider gestational age range compared to the previous study (30–38 and 30–34 weeks of gestation, respectively), which could contribute to these differences [10,15–17]. Concentrations of sST2 were relatively constant until 30 weeks of gestation, after which they increased steadily until the time of delivery due to changes in cytokine concentrations and in the volume of maternal circulation, both of which increased endothelial cell production of sST2 [11].

The mean level of sST2 in the preeclampsia group in our study was higher than in the previous study (85.89 ng/ml vs. 77.82 ng/ml, respectively) [10]. This difference may have been caused by a disproportionate occurrence of early- and late-onset preeclampsia in our sample. Women with early-onset preeclampsia had higher sST2 concentrations than those with late-onset preeclampsia [18,19]. This

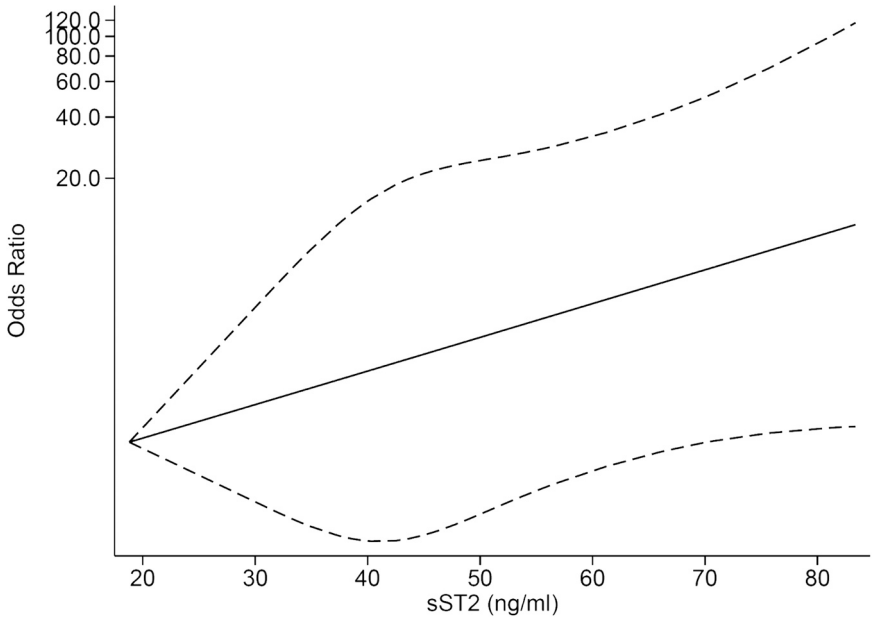

Fig. 3. Dose-response relationship between sST2 and preeclampsia. The dashed lines indicate 95% confidence interval.

phenomenon resulted from higher cytokine concentrations (such as IL-12, TNF-α, and IL-1β) in early- versus late-onset preeclampsia [11].

Results from multivariate analysis further strengthen our conclusion that preeclampsia was the best explanatory variable for the elevated levels of sST2. This was potentially caused by active transportation of sST2, originating from amniotic fluid, across the placenta to the maternal circulation. Evidence suggests that sST2 originating from amniotic fluid participates in the pathogenesis of preeclampsia, while the underlying mechanism of this transport remains unclear [20]. However, the placenta is unlikely to be the sole source of sST2 in preeclamptic pregnancies [10].

Endothelial dysfunction could also contribute to rising levels of sST2 [21]. This could explain another finding in this study, in which sST2 levels were observed to be significantly higher in the third trimester of pregnancy with preeclampsia compared with normal pregnancy. The mechanism of elevated sST2 levels in preeclampsia has been linked with excessive release of anti-angiogenic sFlt-1 (the soluble VEGF receptor) from the oxidatively stressed placenta. Angiogenesis both stimulates and is stimulated by inflammation. Soluble ST2 appears to be an anti-inflammatory agent produced by the placenta in excess amounts throughout the course of preeclampsia [10,22,23]. Other cardiac conditions associated with ventricular hypertrophy and remodeling, another feature of preeclampsia, could increase circulating levels of sST2 derived from cardiomyocytes that are activated by mechanical stress or pro-inflammatory stimuli [24,25].

This study also indicates that sST2 level > 63.66 ng/ml has sensitivity of 50% and specificity of 93.3%, with AUC of 0.78, which may be used to rule in preeclampsia. The dose-response between sST2 level and preeclampsia was linear that indicates a proportional relationship between sST2 level and risk of preeclampsia. This finding was consistent with the pathogenesis and natural history of preeclampsia, as sST2 will pathologically increase in response to increase inflammatory cytokines. As far as we know, sST2 serves as a marker of inflammatory process, and whether sST2 could worsen the inflammatory process remains unclear. We hypothesized that the higher value of sST2, the higher probability of the individual developing preeclampsia and is at higher risk of intracardiac remodeling. The value of 63.66 ng/ml might be useful to determine the cut-off value of sST2 to rule in preeclampsia in daily practice, however, further studies are needed to confirm these findings.

Conclusion

Our study demonstrates the increased levels of sST2 in the third trimesters of both normal and preeclamptic pregnancies. Women with normal pregnancies had higher levels of sST2 compared to normal reference values of sST2, and sST2 levels were significantly increased in preeclamptic pregnancies. We also found preeclampsia to be the best explanatory variable for the elevation of sST2 levels in areas of southeast Asia with an ethnic Malay predominance. We hypothesized that the placenta is responsible for higher-than-normal levels of sST2 in normal pregnancy and that a stressed placenta, as occurs in preeclampsia, secretes even more sST2. Furthermore, the level of sST2 in ethnic Malay may be higher in both normal and preeclampsia patients compared to the previous study. Therefore, the functional role of sST2 in pregnancy and preeclampsia may differ across ethnicities and needs further investigation.

Limitations

This study has limitations. First, our method categorized normal pregnancy based solely on history-taking, which could bias the results. Second, we did not observe the sST2 levels or clinical conditions of patients in their first or second trimesters of pregnancy or their post-partum period, so the prognostic value of sST2 levels can not be evaluated. Third, the relatively small sample size and recruitment of patients from only two referral centers in West Java may not accurately represent the whole population.

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Disclosure statement

Hawani Sasmaya Prameswari, Achmad Fitrah Khalid, Dewi Anggraeni, and Setyorini Irianti declare that they have no conflict of interest in this study.

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