Triggering receptor expressed on myeloid cells (TREM) like transcript-1 (TLT-1) reveals platelet activation in preeclampsia

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Abstract

Triggering receptor expressed on myeloid cells (TREM) like transcript-1 (TLT-1) is a membrane protein receptor found in α-granules of megakaryocytes and platelets. Upon platelet activation TLT-1 is rapidly relocated to the surface of platelets. In plasma, a soluble form of TLT-1 (sTLT-1) is present. Plasma levels of sTLT-1 are significantly elevated in thrombotic diseases. In the present study, we investigated to whether TLT-1 reflects platelet activation in pregnant women with preeclampsia. We studied 30 preeclamptic patients who were matched with 30 normotensive pregnant women and 30 non-pregnant controls. Basal TLT-1, P-selectin, and CD63 expressions on platelets were analyzed with the use of flow-cytometry (FCM). Platelet reactivity was induced by thrombin receptor activation peptide and determined by FCM. Plasma concentrations of sTLT-1 and soluble P-selectin (sP-selectin) were measured by an enzyme-linked immunosorbent assay. Results show that basal platelet expression of TLT-1, P-selectin and CD63 were increased in women with preeclampsia (PE) compared with normotensive pregnant women (NP). Platelets from PE women and NP women were more responsive compared to from nonpregnant women controls (NC), and which was demonstrated by increased expression of TLT-1, P-selectin, and CD63 upon stimulation in vitro. Plasma concentration of sTLT-1 was greater in PE women compared to NP women and NC women. Plasma sP-selectin level was higher in pregnant women than in nonpregnant women, but there were no significant differences between PE and NP women. In summary, our results revealed that platelet activation is prominent in preeclampsia, TLT-1 reflects platelet activation and may be a useful indicator for preeclampsia.

Introduction

Preeclampsia is a serious pregnancy-related complication for both mother and fetus [1] and occurs in 3–5% of all pregnancies [2]. Currently, the diagnosis endorsed by the International Society for the Study of Hypertension in Pregnancy (ISSHP) embraces new onset hypertension (systolic >140 mmHg or diastolic >90 mmHg) accompanied by one or more other features: proteinuria, other maternal organ dysfunction (including liver, kidney, neurological), or hematological involvement, and/or uteroplacental dysfunction, such as fetal growth restriction and/or abnormal Doppler ultrasound findings of utero-placental blood flow [3].

The pathogenesis of preeclampsia is not fully elucidated but tremendous progress has been made over the past decade. The placenta has always been a critical figure in the etiology of preeclampsia because the removal of the placenta is necessary for symptoms to regress [4]. The abnormal spiral artery remodeling has been documented to be the central pathogenic factor in pregnancies complicated by intrauterine growth restriction, gestational hypertension, and preeclampsia [5]. Placental endotheliopathy is the root cause of preeclampsia and disseminates systemically to the maternal circulation. The shift has been made to view preeclampsia as a systemic disease with widespread endothelial damage and the potential to affect future cardiovascular diseases rather than a self-limited occurrence [6].

A protective hypercoagulable state is often developed during late pregnancy and can evolve into a prothrombotic state in patients with preeclampsia [7,8]. Additionally, increasing evidence showed platelet activation plays a vital role in the pathogenesis of preeclampsia [9–11]. Therefore, markers of platelet activation may have a potential for early prediction of preeclampsia.

Presently, flow cytometry (FCM) is the most sensitive technique to detect increased surface exposure of activation antigens on the platelet surface [12–14]. Activated platelets have a different exposure of glycoproteins on the surface of platelets that are measurable by flow cytometry. Platelet degranulation occurs with fusion of platelet granules and lysosomal membranes with the plasma membranes, which could be detected via surface expression of P-selectin (CD62P) [15] and CD63 [16], respectively. The expression of P-selectin and CD63 significantly increased in preeclampsia than that in non-preeclampsia [10,17–20]. However, in
clinical practice, these factors predicted just 30% of women who developed preeclampsia [21]. The test accuracy of these markers restrained the apply in clinical practice [22].

Triggering receptor expressed on myeloid cells (TREM) like transcript-1 (TLT-1) is a membrane protein receptor specific to α-granules of megakaryocytes and platelets. Upon platelet activation TLT-1 is rapidly relocated to the surface of platelets [23,24]. TLT-1 is abundantly present in platelets, with expression levels greater than P-selectin in human (TLT-1,14200 copies; P-selectin, 8900 copies) [25]. TLT-1 facilitates platelet aggregation and mediates platelet interactions with both endothelial cells and neutrophils [26]. Smith et al. reported that surface expression of TLT-1 was a more sensitive marker of platelet activation than P-selectin [27]. A soluble form of TLT-1 (sTLT-1) also exists, the majority of sTLT-1 is shed from the surface of activated platelets, and the remainder is an alternatively splicing isoform stored and released from α-granules [28]. During inflammation, sTLT-1 may mediate hemostasis by enhancing actin polymerization, resulting in increased platelet aggregation and adherence to the endothelium [29]. Plasma levels of sTLT-1 are significantly elevated in sepsis [30] and coronary artery diseases [31]. Despite the proof of the biological functions TLT-1 plays on platelets and sTLT-1 plays in the plasma, there is little information about their role in preeclampsia.

Description of the status of platelets in preeclampsia, the determination of platelet properties (including quantification of basal [16] receptor levels and markers of ongoing activation), and platelet response to a standardized stimulus in vitro is another area of concern.

The primary purpose of our study was to investigate whether TLT-1 reflects platelet activation in pregnant women with preeclampsia. We inspected the TLT-1 expression on platelets in preeclamptic patients, healthy normotensive pregnant women and nonpregnant controls and the plasma concentration of sTLT-1 in these individuals. The sensitive markers of platelet activation, such as P-selectin and CD63, which have been closely linked to the TLT-1 were also studied. We further characterized the reactivity of platelets in the three groups stimulated with thrombin receptor activating peptide-6 (TRAP-6) in vitro.

Methods and Materials

Study Population

Pregnant women were recruited from the obstetrics and gynecology department of The First Affiliated Hospital of Soochow University from May 2020 to January 2021. For this study, we enrolled consecutive women with preeclampsia. Diagnostic criteria for preeclampsia were based on the American College of Obstetricians and Gynecologists Practice Bulletin Number 202 [32] and the Chinese Medical Association of Obstetrics and Gynecologic Guidelines for hypertension in pregnancy (2020) [33]. Exclusion criteria were as follows: (1) usage of anticoagulant or antiplatelet drugs before enrollment; (2) history of hypertension, diabetes, chronic nephropathy, hepatitis, tumors, immune system diseases, and cardiac surgery before pregnancy; (3) history of thrombosis or bleeding disorders; (4) history of recurrent miscarriage; (5) in vitro fertilization and embryo transfer. Thirty preeclamptic patients (PE), thirty healthy normotensive pregnant women (NP) and thirty healthy age-matched nonpregnant controls (NC) were recruited for the case–control study. The normotensive pregnant women attended for routine antenatal care. They were matched for age and gestational age with the preeclamptic women. All pregnant women were followed-up until delivery. Both normotensive pregnant and nonpregnant women denied taking any medication in the previous 2 weeks. The study was approved by the local ethics committee. All participants gave written informed consent.

Reagents and ELISA Kits

Fluorescein isothiocyanate (FITC)-anti-hTREML1/TLT-1 was purchased from R&D (Minneapolis, MN, USA). FITC-labeled IgG1 and phycoerythrin (PE)-labeled IgG1, FITC anti-human P-selectin (CD62P) and PE anti-human CD63 was ordered from Biologend (San Diego, CA, USA). The plasma concentration of sTLT-1 and soluble form of P-selectin (sP-selectin) were detected by enzyme-linked immunosorbent assay (ELISA). Human TLT-1 ELISA kit (EK4453) was purchased from SAB (MD, USA). Human P-Selectin ELISA kit (ab100631) was acquired from Abcam (Cambridge, UK). Thrombin receptor-activating peptide-6 (TRAP-6) was bought from Sigma (St. Louis, MS, USA).

Blood Sampling

Two fasting blood samples were drawn from the antecubital vein without tourniquet through a 21-gauge needle with a vacutainer system. The first sample was collected in a 2.0 mL ethylenediaminetetraacetic acid (EDTA) K2 tube (BD, NE, USA) for FCM assay. The second sample was collected into a 2.7 mL tube containing 0.105 M buffered sodium citrate (BD, Devon, UK). Within 30 minutes after collection, cells were removed by centrifugation for 10 minutes at 1500 g and 20°C. Plasma samples were then divided in 200 uL aliquots, immediately snap frozen in liquid nitrogen and stored at −80°C for further assay. The samples were performed assay within 2 hours of blood collection. All laboratory tests were performed blinded to as case or control status.

Flow Cytometry Assay

Whole blood flow cytometric analysis of clinical samples were modified from Janes et al. [34]. Fifty μL EDTA-anticoagulated whole blood was diluted with 450 μL modified tyrod’s buffer (2.5 mM HEPES, 150 mM NaCl, 2.5 mM KCl, 12 mM NaHCO3, 5.5 mM D-glucose, 1 mM CaCl2, 1 mM MgCl2, pH 7.4). When studying reactivity of platelets in vitro, 49 μL of diluted whole blood were incubated with 1 μL TRAP-6 for 20 minutes at 37°C [10], to reach final concentration of 5 μM. In parallel, same amount of whole blood was incubated with modified tyrod’s buffer as basal status. The mixed suspensions were incubated with FITC- or PE-conjugated antibody for another 20 minutes at room temperature in dark and then mixed with 500 μL of 0.2% (v/v) PFA-PBS (paraformaldehyde (PFA) in phosphate-buffered saline (PBS)) to inhibit further activation, and analyzed through Flow cytometry (FC500, Beckm Couter) with CXP Software Version 2.3 within 2 hours of collection.

The platelet population was identified by light-scatter characteristics and enclosed in an electronic bit-map. Daily control of fluorescence intensity was done with Flow-Set fluorescence microspheres (Coulter Corporation, Miami, FL) according to the manufacturer’s instructions. Five thousand platelets were analyzed from each sample. Results were expressed as percentage of positive platelets, which defines the negative control (isotype control) to 0%. Also, mean fluorescence intensities (MFI) were presented, which were calculated for gated cells. Samples from normotensive pregnant women and nonpregnant women were run in parallel with those from preeclampsia patients.

Enzyme-Linked Immunosorbent Assay

All plasma analyses were subjected to ELISA assay to determine the plasma concentration of sTLT-1 and sP-selectin. Quantitative
analysis of plasma sTLT-1 was performed as the manufacturer’s instructions. Briefly, 100 μL of plasma samples was added to a pre-coated 96 well plate and incubated for 2 hours at 37°C. After 2 hours, the solution was removed without washing. 100 μL of biotinylated anti-TLT-1 antibody was added and incubated for 1 h at 37°C. After 1 h, the solution was discarded and the plate was washed 3 times with 1× washing. After the last wash, remove any remaining washing buffer by inverting the plate and blotting against a paper towel. Then, 100 μL of horse radish peroxidase (HRP)-conjugated streptavidin was pipetted to the wells for another 1 h at 37°C. Repeated wash was performed as above. Then, 100 μL of TMB one-step substrate reagent was added and incubated for 15 minutes at 37°C in the dark. After 15 minutes, 50 μL of stop solution was added and the plate was read at 450 nm using a microplate reader (SpectraMax M2, Molecular devices) with SoftMax Pro software, four-parameter fitting curve was drawn through the standard points. The exact concentration of sP-selectin (diluted 1:50) was detected by ELISA, according to the manufacturer’s instructions. The sensitivity of sTLT-1 assay is 31.25 pg/ml, and inter- and intra-assay coefficients variation (CV) is 12.35% and 4.78%. The sensitivity of sP-selectin assay is 20 pg/mL, and inter- and intra-assay CV is 8.24% and 6.53%.

Statistical Analyses
All data were analyzed with Graph Pad Software version 7.0. The normality of the data was calculated using the D’Agostino & Pearson omnibus normality test. Data were presented as mean with standard deviation (SD) or as median with inter-quartile range (IQR) for normally distributed data and skewed data, respectively. For normally distributed data, one-way ANOVA analysis was used to compare the multiple groups; intergroup comparisons were performed using Tukey’s post-hoc test. Student’s t test was conducted to compare two groups. For skewed data, nonparametric tests Mann-Whitney U test were used to compare the difference among groups. P < .05 was considered statistically significant.

Results
Clinical Characteristics
The Clinical data are depicted in Table I. The three groups were of comparable age, and the pregnant women were of comparable gravidity and parity. As expected, compared to normotensive pregnant women (NP), preeclamptic women (PE) had significantly higher body mass index, systolic and diastolic blood pressure, as well as lower gestational age (P < .01). Pregnant women had significantly lower platelet counts than that in nonpregnant women controls (NC) (P < .01), while there was no significant difference between the preeclampsia and normotensive pregnant women. The median duration of the pregnancy at delivery was 4 weeks shorter for the preeclampsia group than for the NP group (P < .01) as would be expected. Infants delivered by women with preeclampsia had significantly lower birth weights when compared to health pregnancy group (P < .0001).

Table I. Characteristics of patients included in the study.

|                         | Nonpregnant women (n = 30) | Normotensive pregnancy (n = 30) | Preeclampsia (n = 30) |
|-------------------------|-----------------------------|---------------------------------|-----------------------|
| Age (years)             | 29.85 ± 5.57                | 30.87 ± 4.43                    | 30.73 ± 4.46          |
| BMI (kg/m²)             | 20.95 ± 2.28                | 28.26 ± 4.21**                  | 28.99 ± 5.78**        |
| SBP (mmHg)              | 112.90 ± 10.91              | 115.23 ± 9.93                   | 144.83 ± 11.81***     |
| DBP (mmHg)              | 71.80 ± 9.08                | 72.72 ± 7.37                    | 93.73 ± 9.21***       |
| Gravity                 | 1(1–2)                      | 2(1–3)                          | 2(1–3)                |
| Parity                  | 0(0–1)                      | 0(0–1)                          | 0(0–0)                |
| Platelet count (× 10⁹/L) | 239.83 ± 28.21              | 204.75 ± 44.25**                | 194.83 ± 56.03**      |
| Gestational age (Weeks) |                             |                                 |                       |
| at study                | N/A                         | 39(25–40)                       | 34(22–40)             |
| at delivery             | N/A                         | 39(38–40)                       | 35(33–37)             |
| Birth weight (g)        | N/A                         | 3371.67 ± 502.20                | 2318.95 ± 879.36**    |

BMI, body mass index = weight (kg)/height (m)²; SBP, systolic blood pressure; DBP, diastolic blood pressure. Values are presented as mean ± SD or Median (Interquartile range). Analysis by One way ANOVA, t test or Mann–Whitney U test as appropriate. * P < .05; ** P < .01; *** P < .001 vs. Nonpregnant women. # P < .05; ## P < .01; ### P < .0001 vs. Normotensive pregnancy. N/A denotes not applicable.  

Basal Platelet Activation Status
Data related to platelet basal expression of TLT-1, P-selectin and CD63 are presented in Figure 1. TLT-1 expressed on platelet surface in women with preeclampsia was significantly higher when compared to in NP group and in NC group, which was demonstrated both as the percentage of positive platelets and as MFI levels. TLT-1 expression implied both as the percentage of positive platelets and as MFI levels in NP women was significantly higher than in NC women.  

Similar as TLT-1, compared with the nonpregnant women, P-selectin expressed on platelet surface in pregnant patients including preeclampsia women and NP women elevated significantly. The expression of P-selectin in PE women was higher than in NP women indicated as the percentage of positive platelets.  

The expression of CD63 upregulated significantly in women with preeclampsia when compared with in NP women and in NC women, which implied as the percentage of positive platelets. The MFI levels of CD63 in pregnancy women (preeclampsia women and NP women) were significantly higher than in NC women, while there was no difference between in PE and in NP women. The detailed data and statistical conclusion of basal platelet
activation status were summarized in supplementary information 1.

**In Vitro Platelet Reactivity**

Data related to platelet reactivity in vitro are summarized in Table II. On stimulation with 5 μM TRAP-6 in vitro, the median of TLT-1 positive platelets percentage in women with preeclampsia and in NP women was 62.88% and 64.92%, respectively, which were significantly higher than in NC women (44.42%). However, there was no significant difference between in PE women and in NP women. The platelets surface expression of TLT-1 showed as MFI levels in PE women were significantly higher when compared with in NP women and in NC women.

P-selectin expression in pregnant women (preeclampsia women and NP women) was significantly higher than in non-pregnant women, which was indicated as the percentage of positive platelets. There was no significant difference between in PE patients and in NP women.

With regard to CD63 expression, indicated as the percentage of positive platelets, in women with preeclampsia showed significantly higher platelet reactivity than in NC women, while there was no difference between in PE and in NP women.

**Soluble TLT-1 and P-selectin in Plasma**

Individual values of plasma soluble TLT-1 and P-selectin levels are shown in Figure 2. The median concentration of sTLT-1 in the preeclamptic group was 78.95 pg/ml, which significantly higher than in NP women (44.25 pg/ml) and in nonpregnant controls (47.22 pg/ml). The median concentration of sP-selectin in NP women was significantly higher than in NC women (68.51 ng/ml) and in NC women (47.22 ng/ml). The median concentration of sP-selectin in NP women was significantly higher than in NC women (P < .01). The detailed data and statistical conclusion of soluble TLT-1 and P-selectin plasma concentration were summarized in supplementary information 2.

**Receiver Operating Characteristic (ROC) Analysis**

We evaluated the diagnostic values of basal platelet expression of TLT-1 and P-selectin for preeclampsia, which was determined by FCM. Area Under Curve (AUC) of TLT-1, P-selectin was 0.685 (95%CI: 0.552–0.799, sensitivity: 46.67%, specificity: 83.33%) and 0.653 (95%CI: 0.519–0.772, sensitivity: 43.33%, specificity: 86.67%), respectively. Results of Delong’s test suggested that, there was no significant difference of the diagnostic value between TLT-1 and P-selectin. Additionally, the AUC of combined FCM markers was 0.662 (95%CI: 0.552–0.799), sensitivity: 46.67%, specificity: 83.33%, similar to TLT-1 or P-selectin alone, indicated that conjunct FCM markers has no superior diagnostic value than TLT-1 or P-selectin alone.

Furthermore, we performed ROC curve analysis of plasma sTLT-1 and sP-selectin which was detected by ELISA for diagnosis of preeclampsia (Figure 3). Area Under Curve was summarized in Figure 3. Area under curve of soluble TLT-1 and P-selectin was 0.832 (95%CI: 0.731–0.932, sensitivity: 83.33%, specificity: 66.67%) and 0.669 (95%CI: 0.535–0.785, sensitivity: 56.67%, specificity: 76.67%), respectively. While results of Delong’s test suggested that, there was no difference between TLT-1 and P-selectin. Moreover, when a combination model of sTLT-1 and sP-selectin was used, the predictive value improved to an area under the ROC of 0.916 (95%CI: 0.815–0.972), sensitivity: 46.67%, specificity: 83.33%. A combination of sTLT-1 and sP-selectin markers may provide a more useful
screening test than P-selectin ($P < .0001$) alone but not TLT-1 alone ($P = .152$). These data indicated that TLT-1 and P-selectin maybe good markers to reveal platelet activation in preeclampsia.

**Discussion**

To our knowledge, this is the first report of a significantly enhanced platelet surface expression and plasma levels of TLT-1 in preeclamptic patients. Compared with those of normotensive pregnant women and nonpregnant women controls, TLT-1 positive platelets percentage levels and plasma sTLT-1 in preeclamptic patients were markedly increased. Our study demonstrated that TLT-1 reveals platelet activation in preeclampsia, which was demonstrated as both higher TLT-1 positive platelet percent and elevated plasma sTLT-1 in preeclamptic patients when compared with normotensive pregnant women and nonpregnant women controls. Therefore, the elevated expression level of TLT-1 is a novel finding in preeclampsia and essentially supports the concept of platelet activation in this disease. Platelets membrane glycoprotein, expressed on platelets and/or soluble forms of which shed from platelets have been identified as markers for platelet activity in vivo [15,35,36]. As such, TLT-1 joins the growing list of markers of platelet hyperactivity described in this disorder.

Preeclampsia (PE), the leading cause of maternal and fetal morbidity and mortality, is associated with poor fetal growth, intrauterine growth restriction (IUGR) and low birth weight (LBW) [37]. The placenta plays a vital role in the etiology of PE in the mother and her child. PE alters normal placental development during pregnancy resulting in short- and long-term complications for both the mother and the offspring. PE is a leading contributor to IUGR and numerous preclinical models that mimic the pathogenesis of PE demonstrate that IUGR offspring exhibit sex- and age-specific increases in blood pressure and cardiovascular risk [38]. In our finite cohort of 30 preeclamptic women, 16 cases of LBW were reported, during them 6 cases complicated with IUGR.

P-selectin, an adhesion molecule in the secretory granules of platelets, is a sensitive and specific index of platelet activation. Circulating degranulated platelets rapidly lose surface P-selectin to the plasma pool, and hence platelet surface P-selectin is not an ideal marker for the detection of circulating degranulated platelets [39]. P-selectin also presents in Weibel-Palade bodies of endothelial cells [35]. This implies that sP-selectin in plasma can originate from endothelial cells and/or platelets. However, strong correlations between plasma sP-selectin concentration and platelet count as well as α-thromboglobulin [9], and a lack of correlation with endothelial activation markers [11], suggest that plasma sP-selectin most likely originates from platelets. Therefore, soluble P-selectin reflects the continuous activation status of platelets in vivo, may still be a useful marker of platelet activation in clinical settings. Multiple reports have shown significantly enhanced platelet surface expression and plasma levels of sP-selectin in women with preeclampsia compared with normotensive pregnant women [10,11]. Our study found significantly enhanced platelet surface expression and plasma levels of sP-selectin in women with preeclampsia compared with normotensive pregnant. We also found the higher basal P-selectin levels and being more responsive to in vitro agonist stimulations in women with preeclampsia compared with NP women. These results are in keeping with findings of other investigators [10,17,19,40].

CD63 is another classical platelet activation marker. We reported elevated basal state platelet CD63 expression in both
the preeclampsia and normotensive pregnant women compared with in nonpregnant group indicated as higher CD63 positive platelet percent. Our study reconciled the results of previous studies [11,17,18,34], indicated that platelets from women with pregnant differed from platelets from nonpregnant women by expressing higher basal CD63 levels and being more responsive to in vitro agonist stimulation. Nevertheless, in contrast with our study, Holthe and Staff found there was no significant difference of basal CD63 expression between women with preeclampsia and NP women [10]. Differences in the study design possibly can explain this discrepancy.

During the study of the in vitro stimulated platelet response to TRAP-6, additional clues came forward that extended the information, which was yielded by the investigation of the basal platelet activation status alone. In substance, this approach contributed to discrimination between the pregnancy (women with preeclampsia/NP women) and the nonpregnant state, but in the case of TLT-1 also further distinguished the preeclampsia patients from normotensive pregnant women.

Regarding the soluble biomarkers, we found plasma concentration of sTLT-1 in PE group was markedly higher than in normotensive pregnant and nonpregnant women. And at a cutoff value of 46.1 pg/ml, sTLT-1 had sensitivity of 83.3% and specificity of 66.7% for preeclampsia diagnosis. The platelet activity implied as soluble P-selectin concentration between PE patients and NP women showed no significant difference. At a cutoff value of 85.0 ng/ml, soluble P-selectin was found to have sensitivity of 56.7% and specificity of 76.7% for preeclampsia development. These results are in line with the findings of Mari’a E. Chavarr’a et al., they reported that the cutoff value of soluble P-selectin for preeclampsia development was 81.5 ng/ml [41]. Moreover, the combination of sTLT-1 and sP-selectin markers may provide a more useful screening test than sP-selectin alone. The elevated TLT-1 expression levels both on platelet surface and soluble form in plasma further confirmed that higher TLT-1 levels may be a valuable indicator for platelet activation in preeclampsia.

One limitation of our study is based on a small sample of participants. We found the area under curve (AUC) of sTLT-1 in plasma was greater than AUC of sP-selectin, while without significant difference. More cases need to be recruited to verify whether soluble TLT-1 in plasma is preferable to sP-selectin as an indicator for platelet activation in preeclampsia. Furthermore, there are two sub-types including early and late onset preeclampsia, with others almost certainly yet to be identified. It is believed that pathogenetic mechanisms contributes in varying degrees in early and late onset pre-eclampsia. As reported, early and late onset preeclampsia are characterized by different biomarkers [28,32,33]. We didn’t find the difference of concentration of sTLT-1 or sP-selectin between early and late onset preeclampsia patients. Our data were not able to further discriminate 12 cases of early and 18 cases of late onset preeclampsia in this study (unpublished data) for the finite cohort of 30 preeclamptic women. Further research should be undertaken to investigate the potential biomarkers of stratifying by preeclampsia type in future.

In conclusion, our data demonstrated that elevated TLT-1 expression levels both on platelet surface and soluble form in plasma are evident in preeclampsia when compared to normotensive pregnancy. Our results revealed that TLT-1 reflects platelet activation and may be a useful indicator for platelet activation in preeclampsia. These abnormalities of platelets may have implications for the pathogenesis of preeclampsia. Nevertheless, the exact pathogenic significance and clinical validity of TLT-1 in the maternal circulation needs to be better illustrated.
14. Yaw HP, Van Den Helm S, Linden M, Monagle P, Ignjatovic V. Whole blood flow cytometry protocol for the assessment of platelet phenotype, function, and cellular interactions. Platelets. 2021;32(6):786–793. doi:10.1080/09537104.2020.1810222

15. Hsu-Lin S, Fau-Berman CL, Berman CJ Fau - Furie BC, Furie BC Fau - August D, August D, Fau - Furie B, Furie B. A platelet membrane protein expressed during platelet activation and secretion. Studies using a monoclonal antibody specific for thrombin-activated platelets. J Biol Chem. 1984;259(14):9121–9126

16. Nieuwenhuis HK Fau - van Oosterhout JJ, van Oosterhout JF Fau - Rozemuller E, Rozemuller E Fau - van Iwaarden F, van Iwaarden F Fau - Sixma JJ, Sixma JJ. Studies with a monoclonal antibody against activated platelets: evidence that a secreted 53,000-molecular weight lysosome-like granule protein is exposed on the surface of activated platelets in the circulation. Blood. 1987;70(3):838–845

17. Konijnenberg A, van der Post Ja Fau - Mol BW, Mol BW Fau - Schaap MC, Schaap Mc Fau - Lazarov R, Lazarov R, Fau - Bleker OP, Bleker Op Fau - Boer K, Boer K, Fau - Sturk A, Sturk A. Can flow cytometric detection of platelet activation early in pregnancy predict the occurrence of preeclampsia? A prospective study. Am J Obstet Gynecol. 1997;177(2):434–442. doi:10.1016/s0002-9378(97)70212-6

18. Harlow E, Brown MA, Brighton TA, Smith SL, Trickett AE, Kwan YL, Davis GK. Platelet activation in the hypertensive disorders of pregnancy. Am J Obstet Gynecol. 2002;187(3):688–695. doi:10.1067/mob.2002.125766

19. Yoneyama Y, Suzuki S, Sawa R, Miura A, Doi D, Otsubo Y, Araki T. Plasma nitric oxide levels and the expression of P-selectin on platelets in preeclampsia. Am J Obstet Gynecol. 2002;187(3):676–680. doi:10.1067/mob.2002.125764

20. Yoneyama Y, Suzuki S, Sawa R, Kiyokawa Y, Power GG, Araki T. Plasma adenosine levels and P-selectin expression on platelets in preeclampsia. Obstet Gynecol. 2001;97(3):366–370. doi:10.1016/s0029-7844(00)01184-4

21. Leslie K, Thilaganathan B, Papageorgiou A. Early prediction and prevention of pre-eclampsia. Best Pract Res Clin Obstet Gynaecol. 2011;25(3):334–343. doi:10.1016/j.bpobyn.2011.01.002

22. Kleinrouwele CE, Wiegnerink MM, Ris-Stalpers C, Bossuyt PM, van der Post JA, von Dadelszen P, Mol BW, Pajkrt E, Collaboration EC. Accuracy of circulating placental growth factor, vascular endothelial growth factor, soluble fms-like tyrosine kinase 1 and soluble endoglin in the prediction of pre-eclampsia: a systematic review and meta-analysis. BJOG. 2012;119(7):778–787. doi:10.1111/1471-0528.2012.03311.x

23. Branfield S, Washington AV. The enigmatic nature of the triggering receptor expressed in myeloid cells –1 (TLT-1). Platelets. 2021;32(6):753–760. doi:10.1080/09537104.2021.1881948

24. Washington AV, Schubert RL, Quigley L, Disipio T, Feltz R, Cho EH, McVicar DW. A TREM family member, TLT-1, is found exclusively in the alpha-granules of megakaryocytes and platelets. Blood. 2004;104(4):1042–1047. doi:10.1182/blood-2004-01-0315

25. Burkhardt JM, Vaudel M, Gambaryan S, Radau S, Walter U, Martens L, Geiger J, Sickmann A, Zehedi RP. The first comprehensive and quantitative analysis of human platelet protein composition allows the comparative analysis of structural and functional pathways. Blood. 2012;120(15):e73–82. doi:10.1182/blood-2012-04-416594

26. Washington AV, Gibot S, Acevedo I, Gattis J, Quigley L, Feltz R, De La Mota A, Schubert RL, Gomez-Rodriguez J, Cheng J, et al. TREM-like transcript-1 protects against inflammation-associated hemorrhage by facilitating platelet aggregation in mice and humans. J Clin Invest. 2009;119(6):1489–1501. doi:10.1172/jci36175

27. Smith CW, Raslan Z, Parfitt L, Khan AO, Patel P, Senis YA, Mazhari A. TREM-like transcript 1: a more sensitive marker of platelet activation than P-selectin in humans and mice. Blood Adv. 2018;2(16):2072–2078. doi:10.1182/bloodadvances.2018017756

28. Burton GI, Redman CW, Roberts JM, Moffett A. Pre-eclampsia: pathophysiology and clinical implications. BJM. 2019;366(1756–1833):Electronic(12381). doi:10.1136/bmj.i2381

29. Morales J, Villa K, Gattis J, Castro W, Colón K, Lubbков J, Sanabria P, Hunter R, Washington AV. Soluble TLT-1 modulates platelet-endothelial cell interactions and actin polymerization. Blood Coagul Fibrinolysis. 2010;21(3):229–236. doi:10.1097/MBC.0b013e3283581116

30. Esponda O, Morales J, Aguilar A, Gomez M, Washington AV. Clinical studies support a role for trem-like transcript-1 during the progression of sepsis. J Pediatr. 2010;156(3):59–61

31. Shen L, Yang T, Xia K, Yan Z, Tan J, Li L, Qin Y, Shi W. P-selectin (CD62P) and soluble TREM-like transcript-1 (sTLT-1) are associated with coronary artery disease: a case control study. BMC Cardiovasc Disord. 2020;20(1):387. doi:10.1186/s12872-020-01663-2

32. ACOG Practice Bulletin No. 202: gestational hypertension and preeclampsia. Obstet Gynecol. 2019;133(1):1. doi:10.1097/AOG.0000000000003018

33. Hypertensive Disorders in Pregnancy Subgroup CSoO, Gynecology CMA. [Diagnosis and treatment of hypertension and pre-eclampsia in pregnancy: a clinical practice guideline in China2020]. Zhonghua Fu Chan Ke Za Zhi. 2020;55(4):227–238. doi:10.3760/cma.j.cn112141-20200114-00039

34. Janes SL, Goodall AH. Flow cytometric detection of circulating activated platelets and platelet hyper-responsiveness in pre-eclampsia and pregnancy. Clin Sci (Lond). 1994;86(6):731–739. doi:10.1042/cs0860731

35. McEver RP, Beckstead JH, Moore KL, Marshall-Carlson L, Bainton DF. GMP-140, a platelet alpha-granule membrane protein, is also synthesized by vascular endothelial cells and is localized in Weibel-Palade bodies. J Clin Invest. 1989;84(1):92–99. doi:10.1172/JCI114175

36. Dunlop LC, Skinner MP, Bendall LJ, Favaloro EJ, Castaldi PA, Gorman J, Gamble JR, Vadas MA, Berndt MC. Characterization of GMP-140 (P-selectin) as a circulating plasma protein. J Exp Med. 1992;175(4):1147–1150. doi:10.1083/jem.175.4.1147

37. Ashraf UM, Hall DL, Rawls AZ, Alexander BT. Epigenetic processes during preeclampsia and effects on fetal development and chronic health. Clin Sci (Lond). 2021;15;135(19):2307–2327. doi:10.1042/CS20200900

38. Alexander BT. Placental insufficiency leads to development of hypertension in growth-restricted offspring. Hypertension. 2003;41(3):457–462. doi:10.1161/01.HYP.000005434995913.3D

39. Michelson AD, Barnard MR, Hechtman HB, MacGregor H, Connolly RJ, Loscalzo J, Valeri CR. In vivo tracking of platelets: circulating degranulated platelets rapidly lose surface P-selectin but continue to circulate and function. Proc Natl Acad Sci U S A. 1996;93(21):11877–11882. doi:10.1073/pnas.93.21.11877

40. Macey MG, Bevan S, Alam S, Vergheese L, Agrawal S, Beski S, Thrusisingham R, MacCallum PK. Platelet activation and endogenous thrombin potential in pre-eclampsia. Thromb Res. 2010;125(3):e76–81. doi:10.1016/j.thromres.2009.09.013

41. Chavarria ME, Lara-Gonzalez L, Garcia-Paleta Y, Vital-Reyes VS, Reyes A. Adhesion molecules changes at 20 gestation weeks in pregnancies complicated by preeclampsia. Eur J Obstet Gynecol Reprod Biol. 2008;137(2):157–164. doi:10.1016/j.ejogb.2007.06.014