Severe preeclampsia: Are hemostatic and inflammatory parameters associated?

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1. Introduction

Preeclampsia (PE) is a multi-system disorder of human pregnancy characterized by hypertension and proteinuria occurring after the 20th week of pregnancy in women who have had no previous symptoms [1,2]. Clinically, it is important to diagnose the severe form of the disease, in which hypertension and proteinuria are much higher [1]. The only definitive treatment is to deliver the baby and placenta, often prematurely, in the interest of the baby, the mother, or both [1].

PE is associated with deposition of fibrin in microvasculature, which results in placental perfusion compromised, intraterine fetal growth retardation and dysfunction in some maternal organs [3-5]. Symptoms frequently observed in preeclamptic women include headache, blurred vision, and abdominal pain. This disease can progress to eclampsia (characterized by seizures as a sign of affection of the cerebral vessels), HELLP syndrome (hemolysis, elevated liver enzyme, low platelets) or disseminated intravascular coagulation [1,2].

An intensified inflammatory reaction usually occurs in preeclamptic women compared to normotensive pregnancy [6-8]. PE is associated with circulatory disturbances caused by systemic maternal endothelial cell dysfunction and/or activation; however, the causes of such dysfunction are not well understood [9]. Coagulation, fibrinolysis and inflammation are integral parts of the host immune response. Activation of inflammatory and coagulation pathways is important in the pathogenesis of vascular disease and both systems interact strongly, so that coagulation and inflammatory activity mutually modulate each other [10,11]. Such processes appear to be intrinsically related to PE since the disease is associated with endothelial cell dysfunction, increased inflammatory responses and hypercoagulability [3-9].

2. Methods

2.1. Study population

A total of 59 pregnant women with severe PE (sPE), 49 normotensive pregnant and 48 non-pregnant women were selected from Brazil (2009-2011). sPE was defined by systolic blood pressure ≥ 160 mmHg or diastolic blood pressure ≥ 110 mmHg, on ≥ 2 consecutive occasions ≥ 4h apart; and proteinuria ≥ 2 g/L or at least 2+ protein by dipstick. The normotensive pregnant women had systolic/diastolic blood pressure below 120/80 mmHg and no history of hypertension or proteinuria. All studied women were age matched and all
pregnant women were gestational age matched. Non-pregnant women had no clinical and laboratory alterations.

Common exclusion criteria for the 3 groups were chronic hypertension, hemostatic abnormalities, cancer, diabetes, and cardiovascular, autoimmune, renal and hepatic diseases, anticoagulant or corticosteroid therapy. This study was approved by the Ethics Committee at Federal University of Minas Gerais, Brazil, and informed consent was obtained from all participants. The research protocol did not interfere with any medical recommendations or prescriptions.

2.2. Blood sampling

Blood samples were drawn in sodium citrate (0.129 mol/L) in 9:1 volume ratio and EDTA-K3 1.8 mg/mL (Vacuette®). Citrated blood samples were centrifuged at 2500 g for 20 min at 4 °C to obtain plasma. Samples were aliquoted and stored at 70 °C until analysis of D-dimer and plasminogen activator inhibitor type-1. EDTA blood samples were centrifuged at 2500 g for 20 min at 4 °C to obtain the plasma samples. One mL plasma aliquots were stored at −70 °C until use for flow cytometric cytokine measurements.

2.3. Assays

2.3.1. D-Dimer (D-Di) and plasminogen activator inhibitor type-1 (PAI-1)

Specific commercially available enzyme-linked immunosorbent assay (ELISA) Kit Imuclone® D-Dimer (American Diagnostica Inc.) and Kit IMUBIND® Plasma PAI-1 (American Diagnostica) were used according to the manufacturer’s instructions.

2.3.2. Cytokines

Cytokine plasma concentrations were determined using two commercially available kits: Human Th1/Th2 Cytometric Beads Array – CBA (BD Biosciences Pharmingen) for IFN-γ, and Human Inflammation kit for IL-8, IL-6, and TNF-α. The method was carried out as recommended by the manufacturer. Data acquisition and analysis were performed in dual-laser FACScalibur™ flow cytometer (BD Biosciences Pharmingen), using the BD Bioscience CBA software. Results were expressed as mean fluorescence intensity (MFI) for each cytokine.

2.4. Statistical analysis

Statistical analysis was carried out using SPSS (ver. 13.0). Data normality was tested by Shapiro–Wilks test. Comparisons between two groups were made by Student t test for parametric variables and Mann–Whitney for non-parametric variables. A comparison of non-parametric variables was done by Kruskal–Wallis test among three groups. When differences were detected, they were compared in pairs by Mann–Whitney method, followed by Bonferroni correction. Spearman’s correlations were computed to assess correlations with plasma cytokine concentrations and hemostatic parameters. To evaluate the performance of D-Di, PAI-1, IL-8, IL-6 and IFN-γ as a tool for severe PE diagnosis, the area under the receiver–operator characteristic (ROC) curve was calculated. A P < 0.05 was considered statistically significant (Fig. 1).

3. Results

Table 1 summarizes the clinical characteristics of the 156 women enrolled in this study. Severe PE women, normotensive pregnant and non-pregnant women presented similar ages and pre-pregnancy body mass index (BMI) (P = NS). sPE and normotensive pregnant women did not show differences regarding gestational age. As expected, systolic and diastolic blood pressures were significantly higher in women with sPE (P < 0.001 and P < 0.001, respectively), as well as gestational weight gain, when compared to the normotensive pregnant group (P = 0.001).

Hemostatic markers and cytokine concentrations are summarized in Table 2. D-Di and PAI-1 were significantly higher in sPE group as compared to normotensive pregnant women (P < 0.001 and P < 0.001, respectively) or to non-pregnant women (P < 0.001, in both cases). Furthermore, D-Di and PAI-1 were also significantly higher in pregnant women as compared to non-pregnant women (P < 0.001, in both cases).

IL-8, IL-6, and IFN-γ were significantly higher in the sPE group, compared to normotensive pregnant women (P < 0.001, P < 0.001, and P = 0.024, respectively), while only IL-6 and IFN-γ were higher comparing sPE and non-pregnant women (P < 0.001, in both cases). IFN-γ was also significantly higher in normotensive pregnant women as compared to non-pregnant women (P = 0.018). On the other hand, no difference was found for TNF-α comparing the three groups studied (Table 2).

Fig. 2 presents the area under the ROC curve for D-Di, PAI-1, IL-8, IL-6 and IFN-γ and these parameters showed to be able to detect the sPE (P < 0.001, P < 0.001, P = 0.021, and P = 0.020, respectively). D-Di showed an increased area under curve (AUC), above 0.900, proving to be excellent for detecting sPE in the population studied, as well as PAI-1 concentrations, which showed an AUC above 0.800. On the other hand, IL-8 and IL-6 showed to be bad for discriminating sPE (AUC = 0.697 and 0.698, respectively).

Correlation analysis showed a weak positive correlation between D-Di and IL-8 (r = 0.597, P < 0.001) and between PAI-1 and IFN-γ (r = 0.397, P = 0.045) in sPE. No statistical significant correlation was found for normotensive pregnant women (Table 3).

4. Discussion

Hemostatic and inflammatory pathways mutually modulate and integrate the host immune response [10,11]. Preeclamptic women are known to have an increased hypercoagulable state [3–5], as well as a higher inflammatory response [6–8] compared to those with a healthy

Fig. 1. D-Dimer and PAI-1 plasma levels in studied groups. Results are expressed in mean fluorescence intensity (MFI) and data are presented in a box plot format. The lines stretch from the 10th percentile to the upper 90th percentile, highlighting the outliers (●). The median is shown as a line across the box. Statistical analysis was performed by non-parametric Mann–Whitney test. Significant differences at P < 0.05 are highlighted by connecting lines.
pregnancy. Although some laboratory tests are used to monitor pregnant women in risk of PE, as platelets count and abnormal liver enzyme values, the diagnosis is established effectively by measuring blood pressure and proteinuria [1]. Therefore, to enhance our knowledge about the link between hemostasis and inflammation in PE, further monitoring studies are required at different pregnancy stages. One can speculate that interaction between hemostatic and inflammatory could not be occurring simultaneously for all the gestational period.

Our present investigation showed an increase in D-Di concentrations in sPE women compared to normotensive and non-pregnant women. Besides, higher D-Di concentrations were observed in normotensive pregnant women compared to non-pregnant or to non-pregnant women. Furthermore, PAI-1 plasma concentrations were also significantly higher in normotensive pregnant women as compared to non-pregnant women. The PAI-1 AUC was 0.873, revealing that it is also a good test for detecting sPE.

Previous studies reported higher PAI-1 concentrations in pre-eclamptic pregnant women compared to normotensive pregnant women [4,19–21]. It was also demonstrated that increased PAI-1 concentrations were detected preclinically in pregnant women that show early evidence of placental dysfunction, as well as fetal growth restriction [22]. These findings suggest a decrease in fibrinolytic activity in PE.

Fibrinolysis in vivo is tightly regulated and depends on the balance between plasminogen activators (t-PA and uPA) and plasminogen activator inhibitor (PAI-1) [23]. In the third trimester of healthy pregnancy, there is a four to five fold elevation of PAI-1 plasma concentrations, compared to age matched non-pregnant women [24,25]. Moreover, there is a major inhibition of acute endothelial t-PA release in pregnancy, attributable to excess PAI-1 [25]. It leads to a t-PA:PAI-1 ratio reduction, shifting pregnant women toward a prothrombotic state. Endothelial dysfunction may have a central role in activating hemostatic system by upregulating tissue

Table 1
Clinical characteristics of participants.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Non-pregnant</th>
<th>Normotensive pregnant</th>
<th>Severe preeclamptic women</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>25 (22–30)</td>
<td>23 (18–29)</td>
<td>26 (21–29)</td>
<td>NS</td>
</tr>
<tr>
<td>GA (weeks)</td>
<td>32 (29–35)</td>
<td>33 (31–36)</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>21.6 (20.1–25.4)</td>
<td>23.3 (20.9–26.9)</td>
<td>23.2 (21.4–28.4)</td>
<td>NS</td>
</tr>
<tr>
<td>GWG (kg)</td>
<td>8.5 (4.0–12.5)</td>
<td>12.3 (8.7–15.5)</td>
<td></td>
<td>0.001*</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>120 (110–120)</td>
<td>110 (100–110)†</td>
<td>160 (160–180)**</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>80 (65–80)</td>
<td>70 (63–70)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CA: gestational age; GWC: gestational weight gain; SBP: systolic blood pressure; DBP: diastolic blood pressure; BMI: pre-pregnancy body mass index; (–): does not apply. Data are expressed as median (25th–75th centiles).

Mann–Whitney test and Kruskal–Wallis test were performed.
* Statistically significant.
† Non-pregnant × preeclamptic.
‡ Non-pregnant × normotensive pregnant.
§ Normotensive pregnant × preeclamptic women.

Table 2
Hemostatic parameters and cytokine levels.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Non-pregnant</th>
<th>Normotensive pregnant</th>
<th>Severe preeclamptic women</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-Dimer (ng/ml)</td>
<td>116.9 (90.37–204.1)</td>
<td>891.2 (712.9–1080.0)</td>
<td>1641.0 (1226.0–2073.0)</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>(N = 48)</td>
<td>(N = 49)</td>
<td>(N = 99)</td>
<td>(N = 59)</td>
<td></td>
</tr>
<tr>
<td>PAI-1 (ng/ml)</td>
<td>41.70 (26.81–51.43)</td>
<td>201.7 (172.1–250.9)</td>
<td>286.8 (243.7–318.3)</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>(N = 31)</td>
<td>(N = 26)</td>
<td>(N = 28)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-8 (MFI)</td>
<td>2.93 (1.97–3.85)</td>
<td>2.37 (2.08–2.61)</td>
<td>3.52 (2.46–4.61)</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>(N = 22)</td>
<td>(N = 30)</td>
<td>(N = 43)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-6 (MFI)</td>
<td>8.07 (6.41–10.85)</td>
<td>8.43 (7.17–9.91)</td>
<td>13.82 (9.65–28.13)</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>(N = 22)</td>
<td>(N = 30)</td>
<td>(N = 43)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNF-α (MFI)</td>
<td>3.34 (3.60–4.30)</td>
<td>4.00 (3.45–4.55)</td>
<td>4.10 (3.78–5.15)</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>(N = 48)</td>
<td>(N = 37)</td>
<td>(N = 50)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IFN-γ (MFI)</td>
<td>3.54 (2.80–3.96)</td>
<td>3.69 (3.31–4.13)</td>
<td>3.98 (3.58–4.42)</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>(N = 48)</td>
<td>(N = 37)</td>
<td>(N = 50)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

PAI-1: Plasminogen activator inhibitor type-1; IL: interleukin; TNF-α: tumor necrosis factor type alpha; IFN-γ: interferon type gamma; MFI: mean fluorescence intensity; sPE: severe pre-eclamptic women. Data are expressed as median (25th–75th centiles). Mann–Whitney test and Kruskal–Wallis test were performed.
* Statistically significant.
† sPE × non-pregnant women.
‡ sPE × pregnant women.
§ Pregnant women × non-pregnant women.
factor expression and downregulating natural anticoagulant activity. In parallel, the altered balance between both fibrinolytic activators and inhibitors corroborates for increasing hypercoagulability.

Taking together, our data suggest that elevated D-Di concentrations represent an exacerbated production of fibrin in women with sPE. D-Di concentrations reflect both fibrin polymerization and its breakdown in vivo [12] and the high concentrations found in sPE are probably due to fibrin production. However, it can be inferred that D-Di concentrations may be underestimated since higher concentrations of PAI-1 as observed in this study could decrease the efficiency of the fibrinolytic system, since fibrinolytic system seems to be downregulated by higher PAI-1 concentrations.

Corroborating to the idea of an unbalance between fibrinolytic activators and inhibitors, D-Di/PAI-1 ratios in sPE, normotensive pregnant and non-pregnant women were 5.7, 4.4 and 2.8, respectively, confirming the prothrombotic state in women with sPE. A second ratio established between D-Di/PAI-1 for sPE or normotensive pregnant women in relation to non-pregnant women suggests that normotensive pregnant women are 57% (1.57), while sPE is 104% (2.04) more hypercoagulable than non-pregnant women. Such results were expected, since fibrin deposition is usually found in the subendothelium of the glomerulus and in decidual segments of spiral arteries in preeclamptic women [26].

Concerning cytokines, our data showed higher IL-8, IL-6 and IFN-γ concentrations in sPE women compared to normotensive pregnant women, which show a greater inflammation in severe preeclamptic women (Table 2). Elevated IL-6 concentrations in PE have also been observed in a number of studies, as demonstrated in a recent metaanalysis [27].

### Table 3

<table>
<thead>
<tr>
<th>Population</th>
<th>Cytokine</th>
<th>Hemostatic parameter</th>
<th>Spearman's (rho)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normotensive pregnant</td>
<td>IL-8</td>
<td>D-Dimer</td>
<td>0.075</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PAI-1</td>
<td>0.092</td>
</tr>
<tr>
<td></td>
<td>IL-6</td>
<td>D-Dimer</td>
<td>-0.006</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PAI-1</td>
<td>-0.054</td>
</tr>
<tr>
<td></td>
<td>TNF-α</td>
<td>D-Dimer</td>
<td>-0.049</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PAI-1</td>
<td>-0.067</td>
</tr>
<tr>
<td></td>
<td>IFN-γ</td>
<td>D-Dimer</td>
<td>-0.199</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PAI-1</td>
<td>0.062</td>
</tr>
<tr>
<td>Severe preeclamptic women</td>
<td>IL-8</td>
<td>D-Dimer</td>
<td>0.597*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PAI-1</td>
<td>0.190</td>
</tr>
<tr>
<td></td>
<td>IL-6</td>
<td>D-Dimer</td>
<td>0.248</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PAI-1</td>
<td>-0.128</td>
</tr>
<tr>
<td></td>
<td>TNF-α</td>
<td>D-Dimer</td>
<td>0.221</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PAI-1</td>
<td>0.221</td>
</tr>
<tr>
<td></td>
<td>IFN-γ</td>
<td>D-Dimer</td>
<td>0.026</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PAI-1</td>
<td>0.397*</td>
</tr>
</tbody>
</table>

TNF, tumor necrosis factor; IL, interleukin; IFN, interferon.

Correlation analysis performed by the Spearman correlation test.

* Statistically significant difference (P < 0.05).
Pro-inflammatory cytokines can induce functional and structural alterations, including oxidative damage or interference in vascular constriction/relaxation, leading to alterations in vascular integrity, tone and coagulation [28]. Therefore, plasma cytokines have been suspected to be involved in the pathogenesis of PE for a long time [29,30]. It is known that IL-8 is a potent chemoattractant agent produced by activated neutrophils and previous studies also showed high IL-8 concentrations in PE [31–33].

Accordingly to our data, other studies also found high IFN-γ concentrations in PE [32–36]. However, two studies did not find differences in IFN-γ concentrations comparing PE women and normotensive pregnant women [37,38]. Therefore, the role of IFN-γ in the pathophysiology of PE remains to be clarified.

Our data did not show difference in TNF-α comparing the three groups (Table 2). TNF-α is a powerful pro-inflammatory cytokine and it is present in human placental and uterine cells, both early and late in gestation [39]. In agreement, other studies did not find significant difference in TNF-α concentrations comparing PE and normotensive pregnant women [38,40–42]. However, several studies have reported elevated TNF-α plasma concentrations in PE, suggesting that this cytokine is involved in the pathogenesis of this disease [27,43–47]. The lack of consistency may be due to the relatively short half-life of the cytokine, as well as possible transient and episodic release, which may lead to a very considerable variation in its plasma concentrations [38].

In order to evaluate the relationship between hemostasis and inflammation in sPE, correlation analysis among the markers evaluated was performed. Only a weak positive correlation between PAI-1 and IFN-γ was found in sPE (Table 3). Similarly, regarding D-Di and cytokine concentrations, only a weak positive correlation was obtained in sPE (D-Di and IL-8). A previous study showed that coagulation of whole blood in vitro results in a detectable expression of IL-8 [48]. Fibrin can also activate endothelial cells, eliciting the synthesis of IL-6 and/or IL-8 [49]. Thrombin and fibrin can directly stimulate mononuclear cells and endothelial cells, inducing the synthesis of IL-6 or IL-8 [49].

It has been admitted that the endothelium sensitivity to cytokine effects vary among subjects. As a result, normal cytokine concentrations could become injurious in some women, while others could tolerate high concentrations without endothelial lesions. This fact could explain the absent correlation between hemostatic and inflammatory markers obtained in our study [38].

References


5. Conclusion

D-Di and PAI-1 concentrations showed to be important tool for monitoring sPE. However, no important correlation between these hemostatic markers and cytokine concentrations was found as expected, since hemostasis and inflammation are linked and influence each other. Some speculations for the lack of the expected correlations may be done, as the multifactorial characteristics of PE, including the endothelium dysfunction, nitric oxide pathway, renin-angiotensin system and genetic factors, which represent confound factors for the disease understanding. Besides, it is possible that the hemostatic and inflammatory alterations may not be occurring simultaneously, which would prevent the joining of the cytokines and hemostatic markers’ peak. Another possible explanation would be the fact that D-Di, PAI-1 and cytokines were evaluated systemically and the main alterations in PE could be occurring locally in microenvironment uterine. In conclusion, more studies are necessary to improve the knowledge of hemostasis/inflammation in PE.

Acknowledgments

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