Increased serum levels of high mobility group protein B1 and calprotectin in pre-eclampsia

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Abstract
Objective: To determine whether women with pre-eclampsia have serum levels of biomarkers indicative of an elevated systemic inflammatory response.

Method: The present cross-sectional study was conducted among pregnant women either with pre-eclampsia or without pre-eclampsia who were recruited at a single Chinese hospital between August 1, 2016, and April 30, 2017. Eligible women had no history of acute or chronic inflammation. Serum concentrations of high mobility group protein B1 (HMG-1), calprotectin, and Toll-like receptor 4 (TLR4) were measured and compared.

Results: There were 55 patients included (25 with pre-eclampsia and 30 without). The mean serum concentration of calprotectin was 2656.76 ± 1724.56 μg/L in the pre-eclampsia group versus 1877.33 ± 905.69 μg/L in the control group (P=0.036). Among patients with pre-eclampsia, elevated calprotectin levels were positively associated with the duration of hypertension in pregnancy (P=0.031) and were negatively associated with pregnancy duration at delivery (P=0.035). The mean serum concentration of HMG-1 was 72.48 ± 27.57 μg/L in the pre-eclampsia group versus 57.57 ± 20.07 μg/L in the control group (P=0.035). The mean serum concentration of TLR4 was 22.83 ± 8.46 μg/L in the pre-eclampsia group versus 18.83 ± 6.79 μg/L in the control group (P=0.057).

Conclusion: Elevated levels of HMG-1 and calprotectin could reflect an excessive systemic inflammatory response in pre-eclampsia.

KEYWORDS
Calprotectin; Damage-associated molecular patterns; High mobility group protein B1; Inflammation; Pre-eclampsia; Toll-like receptor 4

1 | INTRODUCTION

Pre-eclampsia is a major cause of fetal and maternal morbidity and mortality worldwide.1 Potential etiological factors for this complication of pregnancy include poor placentation, excessive maternal inflammation and immune response, and endothelial dysfunction.2 Several studies have reported that an exaggerated inflammatory response and an imbalanced immune response together play important roles in the development of pre-eclampsia.3–5

Among women with high-risk pregnancies, both damage-associated molecular patterns (DAMPs) released under hypoxic conditions and syncytiotrophoblast microparticles can provoke an
immunologic response and promote persistent inflammatory conditions by activating pattern-recognition receptors. DAMPs are self-molecules primarily released as a result of non-programmed cell death in the first few hours of an injury. They mediate innate immune responses by recruiting and activating immune cells. They are also referred to as alarmins. DAMPs have been suggested to be crucial mediators in pre-eclampsia.

High mobility group protein B1 (HMG-1) and S100 proteins are DAMPs that are hypothesized to be involved in the pathophysiology of pre-eclampsia. As a DNA-binding protein, HMG-1 has been detected in a variety of eukaryotic cell nuclei. This potential immunomodulatory factor can induce cells to produce a variety of cytokines—including IL-6 and IL-8—and acts as a critical extracellular mediator in inflammatory processes. Calprotectin belongs to the S100 calgranulin subfamily and is a 24-kDa heterodimer composed of protein S100-A9 and protein S100-A8. This protein is secreted mainly from neutrophils, has the ability to bind calcium and zinc ions, and represents a biomarker of active inflammation. Calprotectin and HMG-1 are regarded as late inflammatory mediators in many acute and chronic inflammatory responses. These two molecules are reported to be involved in promoting the inflammatory response in acute or chronic infection, autoimmunity, and cancer development. The toll-like receptors are transmembrane proteins that enable extracellular and endosomal recognition of microbes or other infectious components. TLRs exhibit the most diverse repertoire of DAMP ligands amongst the innate immune receptors. Toll-like receptor 4 (TLR4) is involved in the intracellular signaling pathway that leads to activation of the innate immune system.

As an association has been reported between pre-eclampsia and systemic inflammation, the aim of the present study was to evaluate calprotectin, HMG-1, and TLR4 among women with pre-eclampsia and determine whether the serum concentrations of these biomarkers were associated with clinical characteristics.

2 | MATERIALS AND METHODS

The present prospective cross-sectional study was conducted among women at 20–41 weeks of pregnancy either with or without pre-eclampsia who were recruited by advertisement in the Department of Obstetrics and Gynaecology of Beijing Chao-Yang Hospital, Capital Medical University, Beijing, China, between August 1, 2016, and April 30, 2017. The design was approved by the Ethics Committee of Beijing Chao-Yang Hospital, Capital Medical University. Informed consent for peripheral blood sampling was obtained from all participants.

The pre-eclampsia group included women who met the American College of Obstetricians and Gynecologists definition of this condition. Pre-eclampsia was characterized as elevated blood pressure (≥140/90 mm Hg) on two occasions, measured at least 4 hours apart, after 20 weeks of pregnancy in a woman with previously normal blood pressure, plus the presence of proteinuria (≥300 mg per urine collection or a dipstick reading of 1 +). In the absence of proteinuria, pre-eclampsia was defined as new-onset hypertension plus the onset of any of the following factors during pregnancy: platelet count less than 100 000/mL, elevated serum concentrations of creatinine and liver transaminases, pulmonary edema, or cerebral or visual symptoms. The duration of hypertension was calculated from the participants’ prenatal care records.

The control group comprised pregnant women without a diagnosis of pre-eclampsia. Women with full-term healthy pregnancies were recruited from among hospitalized individuals, who were ready to deliver, whereas women with premature healthy pregnancies were recruited when attending outpatient services.

Exclusion criteria for all participants were a medical history of chronic hypertension, renal disease, pre-existing diabetes mellitus, hemostatic disease, and acute or chronic infection. Patients who were in active labor or experienced premature rupture of membranes were also excluded from the present study.

All participants were induced to deliver either vaginally or via cesarean delivery according to their symptoms. Blood sampling was checked up to 48 hours before delivery. Premature healthy pregnancies in the control group were assessed via blood sampling at a matched number of pregnancy weeks before delivery. Clotted blood samples were centrifuged at 800 g for 5 minutes. The serum was collected and stored at −80°C until enzyme-linked immunosorbent assays were performed. Serum concentrations of the three biomarkers were measured using commercial kits according to the manufacturer’s instructions. Calprotectin was evaluated using a Human S100A8/S100A9 Heterodimer Immunoassay kit (R&D Systems, Minneapolis, MN, USA), whereas HMG-1 and TLR4 were assessed using a Human ELISA Kit for HMG-1 and TLR4, respectively (Cloud-Clone, Wuhan, China).

The data were analyzed using SPSS version 17.0 (SPSS, Chicago, IL, USA). Non-parametric statistical analysis was used to verify normal distribution of the data. Continuous variables with normal distribution were presented as mean ± SD; these factors were compared using the Student t test. Variables with non-normal distribution were presented as the median (interquartile range); differences between such variables were calculated using the non-parametric Mann-Whitney U test. Spearman correlation coefficients were also calculated. Statistical significance was defined as a two-sided P<0.05.

3 | RESULTS

The present study included 25 women in the pre-eclampsia group and 30 women in the control group. The characteristics of the participants are outlined in Table 1. The pregnancy duration at sampling varied from 27 to 40 weeks. Pregnancy duration age at delivery and neonatal delivery weight were both lower in the pre-eclampsia group than in the control group (P<0.001).

The serum concentrations of calprotectin, HMG-1, and TLR4 are presented in Figure 1. Levels of calprotectin were 2656.76 ± 1724.56 μg/L in the pre-eclampsia group and 1877.33 ± 905.69 μg/L in the control group (P=0.036). In an exploratory analysis, elevated calprotectin concentration were found to be positively correlated with the duration of...
TABLE 1 Characteristics of pregnant women with or without pre-eclampsia.a

| Characteristic          | Patients with pre-eclampsia (n=25) | Control group (n=30) | P valueb |
|-------------------------|-------------------------------------|----------------------|----------|
| Age, y                  | 32.0 ± 4.6                          | 30.0 ± 2.9           | 0.152    |
| BMI                     | 28.2 ± 2.9                          | 27.0 ± 2.1           | 0.140    |
| Duration of pregnancy, wk |                                     |                      |          |
| At sampling             | 37 (35–38)                          | 37 (35–38)           | 0.791    |
| At delivery             | 37 (35–38)                          | 39 (38–39)           | <0.001   |
| Neonatal delivery weight, g | 2715 ± 1015                        | 3523 ± 338           | <0.001   |
| Blood pressure, mm Hg   |                                     |                      |          |
| Systolic                | 159 ± 13                            | 121 ± 8              | <0.001   |
| Diastolic               | 94 ± 13                             | 70 ± 10              | <0.001   |

Abbreviation: BMI, body mass index (calculated as weight in kilograms divided by the square of height in meters).

aValues are given as mean ± SD or median (interquartile range), unless indicated otherwise.

bThe Student t test was used to assess all characteristics, other than pregnancy duration at sampling and at delivery (non-parametric Mann-Whitney U test); P<0.05 was considered statistically significant.

hypothesis in pregnancy (P=0.031). By contrast, calprotectin levels correlated negatively with pregnancy duration at delivery in the pre-eclampsia group (P=0.035). Similarly, serum concentrations of HMG-1 were elevated in the pre-eclampsia group compared with the control group (72.48 ± 27.57 μg/L vs 57.57 ± 20.07 μg/L; P=0.017).

The serum levels of TLR4 were measured to investigate if this signaling pathway was potentially related to the increased levels of inflammatory mediators in the pre-eclampsia group (Fig. 1). The concentration of TLR4 did not differ significantly between the groups (P=0.057); however, TLR4 concentrations tended to be higher in the pre-eclampsia group than in the control group (22.83 ± 8.46 μg/L vs 18.83 ± 6.79 μg/L). Unlike calprotectin, HMG-1 and TLR4 levels were not associated with either the duration of pregnancy hypertension or pregnancy duration at delivery.

4 | DISCUSSION

The findings of the present study were three-fold. First, serum concentrations of calprotectin and HMG-1 were elevated among women with pre-eclampsia. Second, high calprotectin levels were positively associated with the duration of hypertension in pregnancy but negatively associated with pregnancy duration at delivery in this group. Third, a nonsignificant trend was observed for raised serum TLR4 concentrations among women with pre-eclampsia.

The current finding of elevated serum concentrations of calprotectin in the pre-eclampsia group was consistent with previous research. Braekke et al.16 discovered substantially elevated plasma calprotectin levels among pre-eclamptic women versus normotensive pregnant women; however, no statistically significant between-group differences were found for the levels of calprotectin in amniotic fluid or umbilical venous plasma. In the present study, calprotectin levels increased as the duration of pregnancy hypertension lengthened. To the best of our knowledge, the current analysis was the first to report an association between calprotectin levels and the duration of pregnancy hypertension or pregnancy duration at delivery. One previous study17 found that calprotectin concentration was higher among patients with severe pre-eclampsia than among those with mild pre-eclampsia. Further clarification of the association between calprotectin elevation and the clinical presence of pre-eclampsia has not yet been reported.

As a proinflammatory factor, calprotectin has the ability to trigger an inflammatory reaction.11 Elevated circulating levels of calprotectin are indicative of leukocyte activation and an excessive systemic inflammatory response in pre-eclampsia.18 Sustained inflammation could lead to vascular endothelial injury, immune dysfunction, and worsening inflammatory response. Furthermore, calcium-binding and zinc-binding proteins have previously been shown to inhibit the expression of matrix metalloproteinases, which play an important role in the infiltration of the placental villi and uterine spiral artery remodeling in the early stages of pregnancy.19 Calprotectin displays high affinity binding for calcium, zinc, and manganese.20

FIGURE 1 The serum concentrations of calprotectin (A), HMG-1 (B), and TLR4 (C) among patients with and without pre-eclampsia. Horizontal bars within the scatter plots represent the median values. Student t test was used to compare the groups. Abbreviations: PE, patients with pre-eclampsia; CP, control group of pregnant women without pre-eclampsia; HMG-1, high mobility group protein B1; TLR4, toll-like receptor 4.
Thus, it has been speculated\textsuperscript{19,20} that this molecule is involved in the onset of hypertension during pregnancy. In the present study, the relationship between serum calprotectin levels and pregnancy duration among women with pre-eclampsia could not be clarified. Pre-eclampsia has been shown to be associated with persistent hypoxia in the placenta and shallow placental implantation.\textsuperscript{2,21} However, data are lacking regarding the difference in placental calprotectin expression in a pregnancy complicated by pre-eclampsia versus a healthy pregnancy. Future research should therefore aim to explore this difference.

Many studies have evaluated the role of HMG-1 in pregnancy hypertension but the findings are inconsistent. Wang et al.\textsuperscript{22} reported no statistically significant difference in serum HMG-1 levels between the control and pre-eclampsia groups. By contrast, other studies found a clear increase in serum HMG-1 levels among patients with pre-eclampsia.\textsuperscript{23,24} The present findings were consistent with those of Pradervand et al.\textsuperscript{23} and Naruse et al.\textsuperscript{24} Immunohistochemical analysis revealed that HMG-1 was expressed predominantly in the syncytiotrophoblast of the human placenta.\textsuperscript{20} Cytoplasmic expression of HMG-1 was markedly increased in the syncytiotrophoblast of patients with pre-eclampsia (both severe and early-onset forms) when compared with normotensive pregnant women.\textsuperscript{25} Consequently, it was speculated in the present study that HMG-1 could participate in the pathophysiology of pre-eclampsia and reflect the general exacerbation of inflammation that occurs in this disease.

Placental expression of TLR2 and TLR4 has been well characterized in normal villous tissue, with these proteins predominantly found in the trophoblasts. Some research has suggested that expression of TLR4 in the placenta is increased among patients with pre-eclampsia.\textsuperscript{26} By contrast, Kulikova et al.\textsuperscript{27} found that TLR4 expression in endothelial cells of the terminal villi was 1.3-fold lower among pregnancies with severe pre-eclampsia than among pregnancies not complicated by this condition. Nitsch et al.\textsuperscript{28} used real-time polymerase chain reaction to analyze expression of TLR4 mRNA in maternal peripheral blood neutrophils. These investigators found a statistically significant decrease in expression among pre-eclamptic women when compared with control individuals.\textsuperscript{26} In the present study, although the difference between the two groups was not statistically significant, there was a tendency toward increased serum TLR4 levels among women with pre-eclampsia.

Previously,\textsuperscript{29} HMG-1 has been shown to be modulated by the function of regulatory T cells through the TLR4 pathway and to exacerbate the inflammatory response and organ damage in experimental models of inflammation. Pre-eclampsia is associated with an excessive inflammatory response and immune activation. Thus, the TLR4 pathway is likely to play a role in the pathogenesis of this pregnancy-related condition. Currently, it is unknown whether calprotectin affects the inflammatory and immune responses by activating TLR4. Nonetheless, it would be of considerable interest to explore whether TLR4 might constitute a novel target for pre-eclampsia therapeutic strategies.

A limitation of the present study was the cross-sectional design, which prevented analysis of HMG-1, calprotectin, and TLR4 concentrations for the duration of pregnancy. Future studies should therefore ascertain the expression of DAMPs throughout pregnancy in a large population and determine their roles in pre-eclampsia.

In conclusion, the findings of the present study suggested that DAMPs could play important roles in the pathogenesis of pre-eclampsia. Calprotectin could be a potential biomarker to monitor pre-eclampsia, especially when it develops at an early stage of pregnancy; however, this hypothesis must be confirmed in future studies. Additional research will be necessary to explore the roles of HMG-1, calprotectin, and TLRs in insufficient placenta implantation or in provoking an abnormal immune response. The findings of such studies would aid evaluation of new targets for the diagnosis and treatment of pre-eclampsia.

**AUTHOR CONTRIBUTIONS**

JL contributed to the design of the study, data collection, data analysis, and writing the manuscript. LH contributed to obtaining institutional review board approval, data collection, and revising the manuscript. SW contributed to data collection and reviewing the manuscript. ZZ contributed to the design of the study, data interpretation, and revising the manuscript.

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**CONFLICTS OF INTEREST**

The authors have no conflicts of interest.

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