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ORIGINAL ARTICLE

Significance of platelet endothelial cell adhesion molecule-1 (PECAM-1) and intercellular adhesion molecule-1 (ICAM-1) expressions in preeclamptic placentae

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Abstract Although preeclampsia (PE) is one of the most important problems affecting pregnant women, etiologic factors in its development are still unclear. We aimed to investigate the expression levels of platelet endothelial cell adhesion molecule-1 (PECAM-1) and intercellular adhesion molecule-1 (ICAM-1) in preeclamptic and control healthy placentas. Placental tissue samples were obtained after delivery from patients diagnosed with PE, and from normal term pregnants and analyzed by immunohistochemistry for the expression levels of the two adhesion molecules PECAM-1 and ICAM-1. A strong expression of PECAM-1 in endothelial cells lining the vessel walls of placental villi in placentas of control group was found, but the intensity of PECAM-1 expression was highly reduced in placentas of PE group ($p = 0.017$). Conversely, a strong expression of ICAM-1 was observed in placental villi in PE, significantly higher than that of normal placentas ($p = 0.005$). The findings of a decrease of PECAM-1 expression and an increase of ICAM-1 expression in preeclamptic placenta suggest the existence of functional roles of these adhesion molecules in the pathophysiology of PE, probably by contributing to the reduced trophoblast invasion and the increased vascular damage, respectively. Inhibiting ICAM-1 (i.e., with ICAM-1 monoclonal antibody) and promoting PECAM-1 expression may be good therapeutic approaches to prevent PE symptoms in the future.

Keywords Preeclampsia · Placenta · Immunohistochemistry · Platelet endothelial cell adhesion molecule-1 · Intercellular adhesion molecule-1

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Introduction

Pregnancy-induced hypertension (PIH) and preeclampsia (PE) are complications unique to human gestation which occur in the second half of pregnancy. Over the years, there have been many different theories about etiology and pathogenesis of this disease [1]. However, until now there has been no specific therapy apart from the delivery of the infant. During the establishment of fetoplacental circulation, uterine spiral arteries undergo remodeling. Namely, endothelial cells of spiral arteries are replaced by endovascular extravillous trophoblastic cells and the arterial smooth muscle and elastic component is lost and replaced by fibrinoid [2, 3]. This process terminates in low-resistance, high-output vessels.

Placental blood flow is dependent on humoral and endothelial derived factors due to the lack of autonomic innervation in placental tissue and vessels [4]. Uterine impedance decreases as the gestational age advances up to the 22nd week of pregnancy and remains stable until delivery [5–7]. Failure of this process has been associated

with complications of pregnancy such as PE, and in severe cases, second-trimester miscarriage [8].

PE is associated with significant maternal and perinatal morbidity in those patients with early onset of PE. Although factors such as callicrein–creatinine, coagulation, and vascular function tests, cytokines, and oxidant stress parameters as well as placental peptide hormones have been identified as potential markers for patients at risk for PE, further studies are mandatory to clarify the pathogenesis of PE [9]. In PE, poor placentation causes both oxidative and endoplasmic reticulum stress of the placenta. Antioxidants such as haptoglobin 1–1 have been suggested to have a protective role in PE [10].

Actually, in PE, endothelial damage is seen in the vessels of placental bed. Substantial atherosis characterized by fibrinoid necrosis and leukocyte–macrophage infiltration can also be seen in spiral arteries [11]. These findings suggest that impaired trophoblast invasion may play an important role in the pathogenesis of PE. And adhesion molecules are contributing to this invasion process.

Moreover, in PE both platelet and neutrophil activation can be seen. Platelets can promote vascular damage and obstruction leading to tissue ischemia and further damage [12]. Many substances mediating vascular damage and vasoconstriction are released from activated neutrophils [13]. Recruitment and aggregation of neutrophils and platelets are partially mediated by cell adhesion molecules expressed on the maternal endothelium and at the uteroplacental bed. In addition, abnormally shallow invasion by trophoblastic cells, characteristic of PE, is associated with abnormal expression of adhesion molecules on trophoblastic cells [14]. Among the major endothelial adhesion molecules expressed on endothelium involved in neutrophil and platelet activation are platelet endothelial cell adhesion molecule-1 (PECAM-1) and intercellular adhesion molecule-1 (ICAM-1). Interestingly, the influx of immune cells into the placental villi appears to be mediated by ICAM-1 [15].

ICAM-1 (CD54) is a 95 kDa member of the Ig superfamily found on lymphocytes, vascular endothelium, high endothelial venules, epithelial cells, macrophages, and dendritic cells [16, 17]. Its expression is up-regulated upon stimulation by inflammatory mediators such as cytokines and subsequently facilitates a selective recruitment of leukocytes in a variety of pathological states [16, 18]. ICAM-1-mediated intercellular adhesion events, e.g., the firm adhesion of T cells to epithelial, endothelial, or antigen presenting cells, can be blocked by injection of mAb against ICAM-1 in vitro and in vivo [17–19].

On the other hand, PECAM-1 (CD31) is a 130 kDa member of the immunoglobulin superfamily that is expressed on the surface of circulating platelets, monocytes, neutrophils, and particular T-cell subsets [20]. It is also a major constituent of the endothelial cell intercellular junction and because of this cellular expression pattern, PECAM-1 is implicated in several functions, including transendothelial migration of leukocytes and angiogenesis [21].

We considered PECAM-1 and ICAM-1 as causative factors involved in the development of pathologic changes in preeclamptic pregnant women. To our knowledge, there are insufficient data about their exact role in PE and their expression levels in placental villi. So, we aimed to investigate a possible role of these cell adhesion molecules in preeclampsia by documenting whether this disease is associated with altered expression in immunohistochemical staining of the above adhesion molecules in the endothelium of the vessels within placental villi.

Methods

Patients

The study had the approval of the local ethical committee of the Kafkas University, Kars, Turkey and informed consent from the patients was obtained. 20 pregnant women who were followed up and underwent delivery at Kars Maternal Hospital were enrolled into the study as two groups. Control group included ten healthy pregnants with a normal course of pregnancy, and PE group included ten pregnants diagnosed with PE after 20 weeks of gestation who had previously normal blood pressure. The characteristics of patients in both groups were as follows: The ages of pregnants in the control group were between 20 and 32 and in PE group between 20 and 43 years of age. No systemic diseases were present in both group of pregnants. Intrauterine growth retardation was not present in any of the fetus. All patients were followed up until delivery and their gestational age at delivery and birth weights of infants were noted.

While selecting patients for control group, the pregnants should have normal blood pressure on two occasions daily, at least 6 h apart, measured for a 1 week period of time and no proteinuria was detected on a urine dipstick test. On the other hand, in PE group, for the diagnosis of PE, both hypertension and proteinuria were evaluated. PE was diagnosed as the presence of hypertension (blood pressure $\geq$ 140/90 mmHg) on two occasions, at least 6 h apart, and the presence of proteinuria defined as 0.3 g or more of protein in a 24 h urine collection (usually corresponds with 1+ or greater on a urine dipstick test), but without evidence of end-organ damage in the patient. All patients in PE group were getting magnesium sulfate treatment to prevent the occurence of seizures.

Tissue samples

Placental tissues were taken from 20 pregnant women immediately after delivery. Paraffin blocks were obtained from placental tissue specimens and immunohistochemical staining was performed at the Department of Histology and Embryology at Faculty of Veterinary Medicine, Kafkas University.

Immunohistochemistry

The streptavidin–biotin–peroxidase method was performed for immunohistochemical staining. For each case, representative blocks were selected. Four micrometer thick sections were cut, deparaffinized with xylene and ethanol. To retrieve the antigen, the deparaffinized slides were first heated (at 98°C) for 20 min in 10 mM citrate buffer (pH 6.0) for each PECAM-1/CD31 and ICAM-1 followed by cooling at room temperature for 20 min and washing twice with phosphate-buffered saline (PBS). All sections were incubated with 30 ml/l hydrogen peroxide for 10 min. to inhibit endogenous peroxidase then washed twice with PBS. After incubation with ultra V Bloc, treatment with primary antibody at room temperature was performed using mouse monoclonal antibody against ICAM-1 (Santa Cruz) and rat monoclonal antibody against PECAM-1 (Santa Cruz) at a dilution of 1/100 for 60 min. After washing with PBS, sections were incubated with biotinylated goat anti-polyvalent and streptavidin peroxidase each for 20 min at room temperature. Following PBS washing, the color was developed with 3,3′-diaminobenzidine (DAB) Chromogen (Lab Vision, Thermoscientific) for 5–15 min at room temperature and counterstained with Mayer hematoxylin for 30 s.

Assessment of PECAM-1 and ICAM-1 staining

Expression levels for both PECAM-1 and ICAM-1 were evaluated using a semiquantitative score (graded as 0 = no, 1 = weak, 2 = moderate, and 3 = strong staining) according to the intensity and distribution patterns of the staining reaction and without knowing the pathological evaluation, the diagnosis of each specimen. Cytoplasmic staining was defined as positive in endothelial cells which are lining the vessel walls within placental villi. The endothelium of stem villi, intermediate villi, and terminal villi were all evaluated.

An estimate of the percentage of immunoreactive cells was determined using a score of 0–3 (0: 0–4% cells stained; 1: 5–29% cells stained; 2: 30–59% cells stained; and 3: 60–100% cells stained). The staining intensity was scored as 0–3 (0, negative; 1, weak; 2, moderate; 3, strong). Values for the quantity and staining intensity scores were then multiplied giving results that ranged from 0 to 9.

The expression levels of PECAM-1 and ICAM-1 in endothelial cells were reported according to the following scoring criteria: grade 0 (score 0); grade 1 (scores 1–3); grade 2 (scores 4–6); grade 3 (scores 7–9) [22, 23].

The mean values of staining grades for each molecular markers were compared between control and preeclamptic groups.

Statistical analysis

Statistical analysis was performed by SPSS 11.0 for Windows software. The immunohistochemical data were reported as mean ± SEM. Comparison of the means between two groups were determined using the nonparametrical Mann–Whitney U test. A p value of <0.05 was considered significant.

Findings

Clinical findings

The mode of delivery was as follows; 5/10 in control group and 10/10 women in PE group went to cesarean section. No statistically significant difference of staining grades was detected depending on the way of delivery in control group. The mean date of delivery was 38.9 ± 3 weeks of gestation in control group and 37 ± 2.2 weeks of gestation in PE group with an insignificant difference ($p > 0.05$) between two groups. The mean birth weight was 2.89 ± 0.4 kg in control group, and 3.38 ± 0.3 kg in PE group with a statistically significant difference ($p < 0.05$) between two groups.

Immunohistochemical PECAM-1 expression

We found a strong immunohistochemical expression of PECAM-1 in normal (control) endothelial cells which are lining the vessel walls of placental villi (Fig. 1a) with a mean value of 2.4 ± 0.2 staining grade, but the intensity of the PECAM-1 expression was highly reduced in preeclamptic placentas (Fig. 1b) with a mean value of 1.1 ± 0.3 (Table 1). Difference between PECAM-1 expression in the endothelial cells of normal and preeclamptic placentas was statistically significant ($p = 0.017$).

Immunohistochemical ICAM-1 expression

In control placentas, the mean value of the staining grade of ICAM-1 expressed in endothelial cells which are lining

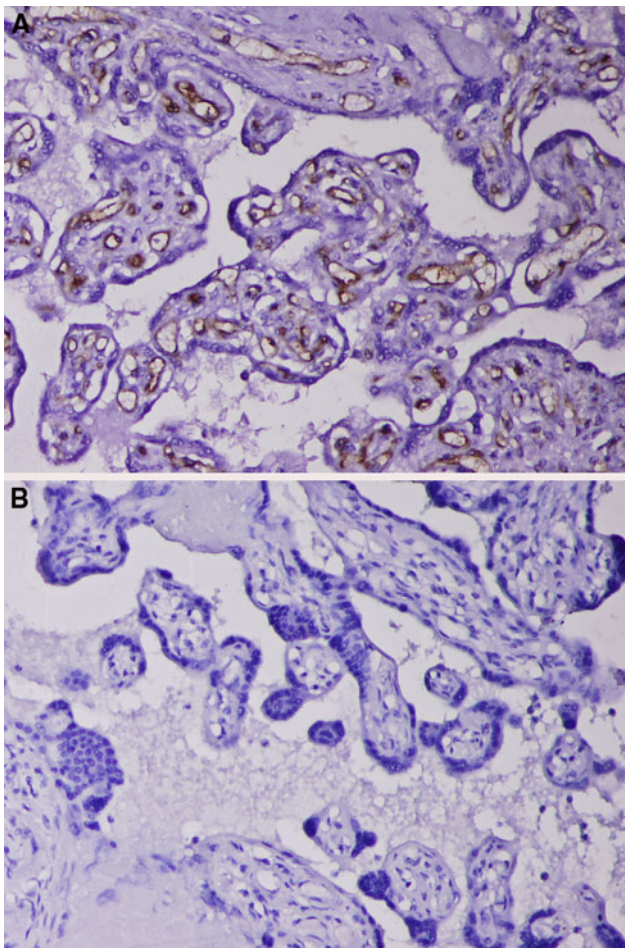

Fig. 1 **a** Moderate immunostaining with PECAM-1 in endothelial cells of preeclamptic placental villi of control (×200). **b** No immunostaining with PECAM-1 in endothelial cells of preeclamptic placental villi of control (×200). Note that PECAM-1 expression in endothelial cells is significantly lower in preeclamptic placenta

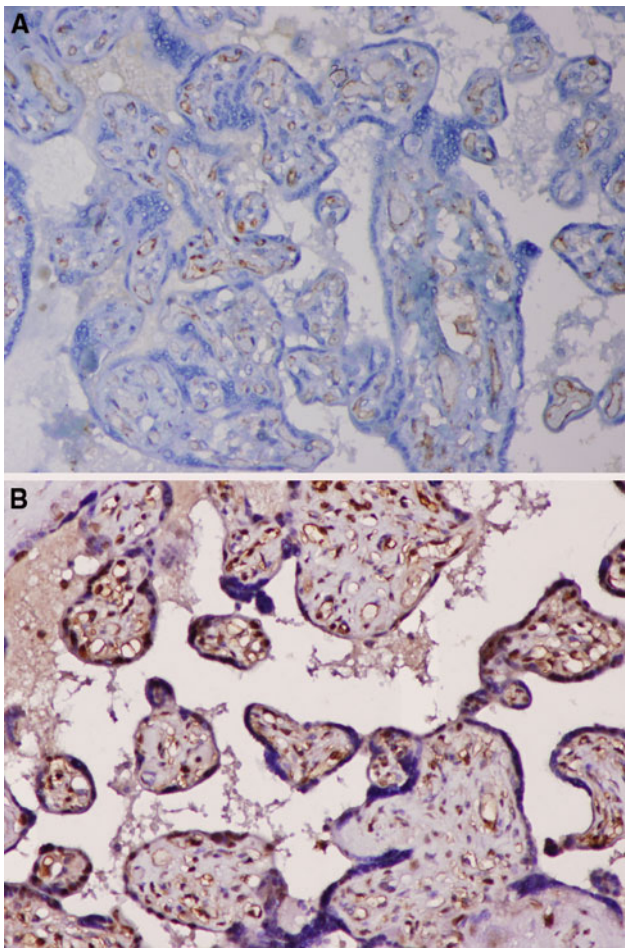

Fig. 2 **a** Mild immunostaining with ICAM-1 in endothelial cells of preeclamptic placental villi of control (×200). **b** Strong immunostaining with ICAM-1 in endothelial cells of preeclamptic placental villi (×200). Note that ICAM-1 expression in endothelial cells is significantly higher in preeclamptic placenta

Table 1 The mean ± SEM values of PECAM-1 and ICAM-1 expressions in control group and preeclamptic group and the mean value differences between two groups that are significant at the aforementioned p levels

	Control group ($n = 10$)	Preeclampsia group ($n = 10$)	Control-preeclampsia: p values
PECAM-1	2.4 ± 0.2	1.1 ± 0.3	0.017
ICAM-1	2.1 ± 0.2	3 ± 0.0	0.005

the vessel walls of placental villi (Fig. 2a) was found to be 2.1 ± 0.2 (Table 1). On the other hand, a strong expression of ICAM-1 was observed with a mean value of 3 ± 0.0 staining grade (Table 1) in endothelial cells of preeclamptic placentas (Fig. 2b), significantly higher than that of normal placentas ($p = 0.005$).

Discussion

Although PE is one of the most important problems affecting pregnant women, etiologic factors in its development are still unclear and many investigations are ongoing to explore the pathogenesis of PE. This disease is a mainly vascular disease of pregnancy, and impaired trophoblast invasion probably plays an important role in the pathogenesis of PE. In this context, in our previous study, we investigated the expression levels of cyclooxygenase-2 (COX-2), tumor necrosis factor-α (TNF-α), and inducible NO synthase (iNOS) in preeclamptic placentas in which we found altered levels of COX-2 and iNOS in PE compared to control, probably by contributing to the reduced placental blood flow and increased resistance to flow in the fetomaternal circulation [24].

Recent literature show that platelets and neutrophils are also involved in the pathophysiology of PE by contributing

to maternal placental vascular damage. And, recruitment of these cells is probably mediated by cell adhesion molecules [25]. For instance, the influx of immune cells into the villi was suggested to be mediated by ICAM-1 [15]. Adhesion molecules are known to be effective in the normal development of a pregnancy, as well, and the analysis of adhesion molecules in PE would provide useful information for clarifying the physiopathology of PE. Thus, we planned to investigate the expressions of two molecular markers related to platelet/immune cell functions in placental villi.

In our study, we demonstrated a significant difference for PECAM-1 and ICAM-1 expressions between the placental villi of healthy pregnant women and the placental villi of women with PE, suggesting the existence of functional roles of these adhesion molecules in the pathophysiology of PE. Namely, higher ICAM-1 and lower PECAM-1 levels were detected in preeclamptic placentas compared to controls.

In our study, a strong expression of ICAM-1 was observed in preeclamptic placental villi, significantly higher than that of normal placentas ($p = 0.005$). Conveniently, Wilczyński et al. [26] found that PE is accompanied by overexpression of ICAM-1 on peripheral blood and decidual lymphocytes. Aliefendioglu et al. [27] and Kim et al. [28] also found that soluble ICAM-1 levels in maternal serum were higher in PE group compared with control group. Interestingly, in another study, serum ICAM-1 concentration was found to be significantly correlated with C-reactive protein and malondialdehyde levels in preeclamptic patients, which implies that recruitment and adhesion of leukocytes to endothelial cells are central features of the generalized intravascular inflammatory reaction and oxidative stress observed in PE [29]. Furthermore, a recent study supporting our findings by Wang et al. showed that anti-ICAM-1 monoclonal antibody (mAb) treatment significantly decreased the levels of blood pressure, urinary protein, maternal blood urea nitrogen (BUN), creatinine and uric acid comparing with untreated preeclamptic rats. And the antibody therapy significantly improved pregnancy outcomes. The authors proposed anti-ICAM-1 mAb therapy as a promising choice for PE [30].

Moreover, sera from PE patients significantly increased ICAM-1 expression on trophoblasts compared to sera from normal pregnant women. TNF-α also enhanced ICAM-1 expression on trophoblasts through nuclear factor-kappaB activation; all suggesting that ICAM-1 expressed on trophoblasts is involved in PE pathogenesis and is regulated by cytokines. Actually, TNF-α is a cytokine that is considered to contribute to endothelial dysfunction. Moreover, many studies showed that serum TNF-α levels were significantly higher in PE than in normal pregnancies [25]. Thus, the increased ICAM-1 expression may in part be attributable to the increased TNF-α in sera of PE patients.

Contrasting with our results, Tziotis reported that ICAM-1 is expressed in the placental bed of normal and preeclamptic pregnancies, with no difference between PE and control [31]. Again, Jaakkola et al. [32] reported that the expression of ICAM-1 was found to be unchanged in placental bed of PE when compared to normal. Lyall et al. [33] reported that immunostaining for ICAM-1 was localized mainly to the endothelium of villi with no difference in ICAM-1 expression in placentae between normal pregnancies and the ones complicated by PE. But when we consider our findings and the supporting literature, expression of ICAM-1 in placental villi of PE is consistent with an abnormal pathological role probably by affecting vascular function.

In this context, it is convenient to mention here that placental angiogenesis is impaired during PE, and the ischemic placenta has been shown to release factors, including the anti-angiogenic molecules soluble fms-like tyrosine kinase 1 (sFlt1) [34] and soluble endoglin (sEng) [35], that result in vasoconstriction and the end-organ damage seen in the mother [36]. The placentas from preeclamptic women have been shown to produce higher concentrations of sFlt-1 in vitro compared to normal controls [37, 38] similiar to our findings of higher ICAM-1 levels. Excess placental sFlt-1 may contribute to endothelial dysfunction, hypertension, and proteinuria in PE [39]. sEng has been shown to inhibit transforming growth factor (TGF)-β1 receptor binding leading to dysregulation of TGF-β1 signaling in the vasculature [40]. The molecular mechanisms that regulate the release of sFlt1 and sEng may also regulate ICAM/PECAM release from the preeclamptic placenta. However, the upstream regulators of these anti-angiogenic proteins and adhesion molecules remain uncertain, and should be further investigated.

On the other hand, in contrast with that of ICAM-1, we found that PECAM-1 activity was significantly lower in placental villi of PE patients than that of control healthy patients ($p = 0.001$). Conveniently, it was reported that in PE, cytotrophoblasts fail to express PECAM-1 and that failure to express endothelial cell adhesion molecules may account for failed trophoblast invasion [41].

Actually, during early human pregnancy, extravillous cytotrophoblasts invade the uterus and spiral arteries transform into large vessels of low resistance. Failure of trophoblast invasion and spiral artery transformation occurs in PE and it seems that PECAM-1 expression has important roles in this process.

Conversely, Lyall et al. [33] reported that there were no differences in PECAM-1 expression in placentae between normal pregnancies and the ones complicated by PE. Therefore, there are some conflicting results about the expressions of these adhesion molecules. Actually, these conflicting results may depend on the differences in

methodologies, interpretation of the data, the hormonal differences of individuals, and the region of the placenta being examined, etc. But when we consider our findings and the supporting literature, expression of PECAM-1 in placental villi of PE is consistent with a pathological role probably by impairing trophoblast invasion.

Another fact to mention here is that the patients in our PE group were getting magnesium sulfate treatment to prevent the occurence of seizures. So we questioned whether the expression levels of cell adhesion molecules would have been affected by magnesium administration. Magnesium is well-known to affect blood pressure by modulating vascular tone and reactivity. It acts as a calcium channel antagonist, it stimulates production of vasodilator prostacyclins and nitric oxide and it alters vascular responses to vasoactive agonists [42].

In a recent study, the effects of magnesium were studied in human umbilical arteries, stem villous arteries, and maternal intramyometrial arteries, reporting that the relaxant effect of magnesium did not seem to be mediated through the endothelium [43]. Moreover, in another study magnesium did not affect trophoblast prostanoid production, and did not alter the media levels of angiotensin II, endothelin-1, or leukotriene B4 [44]. Thus, magnesium likely decreases seizure risk in PE by a mechanism other than altering mediators of arterial remodeling. But on the other hand, a recent study by Amash et al. [45] reported that magnesium sulfate exposure of preeclamptic placentas, but not normal placentas, showed significantly decreased TNF-α levels in the maternal circulations. As we mentioned above, the increased ICAM-1 expression may be attributable to the increased TNF-α in sera of PE patients. We may adapt these study results to our own results by suggesting this hypothesis; the increase in the ICAM-1 expression would have been much more if magnesium sulfate was not applied to our PE patients. Nevertheless, further investigations are needed to come to a certain conclusion.

Finally, as clinical findings in our study, the mean birth weight of infants was found to be significantly lower in PE group than control ($p < 0{,}05$). This may be attributable to the lower mean date of delivery seen in our PE group as 37 ± 2.2 weeks. Supporting this, Xiong et al. [46] reported that birth weights were significantly lower among mothers with PE who delivered at $\leq$37 weeks, with an average difference of -352.5 g. Although PE significantly increases the risk of low birth weight and small for gestational age (SGA) babies who delivered at $\leq$37 weeks, PE also increases the risk of high birth weight and large for gestational age (LGA) babies who delivered at >37 weeks. The phenomenon of LGA and high birth weight infants born to preeclamptic patients may be the result of earlier growth-enhancing effects by an increased uteroplacental blood flow due to higher blood pressure [46].

On the whole, we conclude that the findings of a decrease of PECAM-1 expression and an increase of ICAM-1 expression in preeclamptic placenta suggest the existence of functional roles of these adhesion molecules, which contribute to failure of trophoblast invasion and spiral artery transformation and the vascular damage involving platelets and neutrophils in maternal placenta as characteristics of the pathophysiology of PE. In this context, PECAM enhancing therapies and anti-ICAM mAb treatment may be potential candidates to prevent PE. And more investigations are mandatory to explore the therapeutic aspects of these two adhesion molecules.

Conflict of interest The authors report no declarations of interest.

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