Assessment of Urinary Podocalyxin as a Novel Biomarker for the Diagnosis of Decidual Vasculopathy in Preeclampsia: a Case-Control Study

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Abstract

Background: Preeclampsia is a hypertension-related disorder in pregnancy that increases the risk of maternal and fetal mortality and morbidity. The incidence is estimated to be 10% globally. Pre-eclampsia is thought to develop as a result of decidual vasculopathy (DV), which is characterized by aberrant remodeling of uterine spiral arteries. The risk factors and pathophysiology of preeclampsia are well established; however, reliable biomarkers that can help with the early detection of high-risk individuals are currently needed. We aimed to assess podocalyxin as a potential biomarker of decidual vasculopathy.

Methodology: 18 pregnant women with preeclampsia and 2 controls were recruited. H&E Staining and Immunohistochemistry were performed on paraffin-block placenta tissues that were obtained from 8 cases and 1 control.

Results: All participants were in their third trimester of gestation. Poor remodeling of the decidual vasculature, evidenced by hypertrophy of the tunica media and deranged endothelium, was observed in the case group as compared with the control group. Immunohistochemistry showed strong staining in the placenta tissues of the case group but mild staining for the control group.

Conclusion: The histological evidence of aberrant vascular remodeling in the case group correlated with strong immunohistochemical staining for podocalyxin.

Introduction

Preeclampsia is defined by the development of a new onset of hypertension (≥ 140/90 mmHg) and proteinuria after 20 weeks of pregnancy, either with or without end-organ failure (Mansilla et al., 2018). The incidence is estimated to be 10% of pregnancies worldwide, and it

contributes to about 15% of global maternal mortality. Preeclampsia can lead to both maternal and fetal complications, such as maternal renal failure, liver failure, stroke, stillbirth, preterm birth, and intrauterine growth restriction (Boeldt & Bird, 2017). Despite improvements in our knowledge of the pathophysiology of preeclampsia, the absence of reliable biomarkers and the variability in clinical presentation make early screening, diagnosis, and prediction difficult (Rana et al., 2019.).

The pathogenesis of pre-eclampsia starts in the placental vasculature. During pregnancy, to ensure effective perfusion and exchange of substances between the fetus and the maternal circulations, vasodilation of the spiral vessels occurs with less resistance to blood flow. These occur in large blood vessels without smooth muscles (Hecht et al., 2016; Stevens et al., 2020). Decidual vasculopathy describes aberrant alterations or pathology in the blood vessels of the decidua during pregnancy. There are several presentations of decidual vasculopathy, including fibrinoid necrosis, vascular sclerosis, and thrombosis. In delivered fetal membranes, the spiral arteries have distinct pathological alterations at the basal decidua and in the decidua parietalis in preeclampsia. Perivascular lymphocytic infiltration, fibrinoid necrosis, and integration of foam cells into the vessel wall are characteristic morphological abnormalities. Vascular distention is observed, along with endothelium damage, fibrinoid necrosis of the media, and fibrin accumulation. Fibrinoid necrosis is decidual vasculopathy's last stage (Hecht et al., 2016; Stevens et al., 2020).

Podocalyxin is a large membrane glycoprotein that covers the surface of podocytes. The epithelial layer of the vasculature, mesothelium, certain nerves, some blood-producing progenitor cells, some malignancies (glioblastomas, leukemias, germ cell cancers), and the placenta all express podocalyxin. Damage to podocytes and endotheliosis leads to the release of

podocalyxin into the urine. Although the levels of urinary podocalyxin increase in labor and hematuria, it may be used as a biomarker for diagnosing among pregnant women who are not in labor or have hematuria (De Franco et al., 2022; Uzun et al., 2022). Compared to women who were pregnant normally, preeclampsia women's serum podocalyxin levels were statistically considerably higher (Nikolov et al., 2022; Wang et al., 2021). Podocalyxin has a 90% sensitivity and 98% specificity in predicting preeclampsia (Uzun et al., 2022).

Methods

Participants Recruitment:

Pregnant women at ≥ 20 weeks of gestation diagnosed with preeclampsia and admitted to the Obstetrics' pathology unit of the Mother and Child Hospital (UMC) were recruited as the case group. 18 participants enrolled in the study as cases. Two pregnant women without pre-eclampsia were recruited as the control group. Both verbal and written consent were obtained from the participants before their recruitment.

Inclusion and exclusion criteria:

The inclusion criteria were pregnant women with high BP of ≥140/90 mmHg, which started at or after week 20 of gestation, in addition to at least one of the following: proteinuria, edema of the lungs, edema of the feet, change in mental status, and HELLP (Hemolysis, Elevated Liver enzymes and Low Platelets) syndrome. The exclusion criteria were; all pregnant women before 20 weeks of gestation or with the following conditions at any gestational age: primary hypertension before pregnancy, diabetes mellitus, malignancy, human immunodeficiency virus infection, hepatitis, autoimmune conditions, polycystic ovarian syndrome, kidney diseases, transplanted organ recipients, sickle cell disease, human papilloma

virus infection and patients who were only hypertensive without other features such as proteinuria, low platelets, acute kidney injury, hepatic dysfunction and central nervous system manifestations.

Sample Collection and Processing:

Early morning clean-catch urine was taken from participants. The urine was centrifuged for 10minutes at a temperature of 4 degrees Celsius, and a rotation speed of 2000RPM. The centrifuged urine was sieved with a syringe filter 0.45µm, stored in a urine collection and preservation tube, and kept in a freezer at -20 degrees Celsius.

Seven placentas of cases and 1 placenta of controls were collected at delivery. Paraffin blocks of those placentas were prepared and stored for immunohistochemistry and H&E staining. Immunohistochemistry was done using monoclonal antibody (3D3), manufacturer: Thermo Fisher Scientific, with a catalog number: 39-3800, and Goat anti-mouse IgG secondary antibodies (H+L), HRP with catalog number: 31430. All recommended protocols by the manufacturer were observed. H&E staining was also performed on the blocks under standard recommendations. Vasculopathy was assessed in H&E slides, and the expression of podocalyxin was assessed in immunohistochemically stained placenta blocks using a light microscope.
ELISA: Permission to bring serum samples to the NUSOM lab is still pending.

Results

Table 1. Sociodemographic and Clinical Characteristics of Participants

Variable	*Controls (n = 2)*	*Cases (n = 18)*	*p-value*
Age (years)	33.5 ± 0.7	33.4 ± 5.3	0.98
BMI (kg/m²)	30.7 ± 5.7	31.4 ± 6.1	0.87
Gestational age	35.5 ± 0.7	36.2 ± 2.9	0.75
Gravidity category			0.58
- Gravida 1	0 (0.0%)	2 (11.1%)	
- Gravida 2	0 (0.0%)	6 (33.3%)	
- Gravida 3	1 (50.0%)	3 (16.7%)	
- Gravida >3	1 (50.0%)	7 (38.9%)	

Table 1 summarizes the clinical and demographic features of both groups. The BMI and mean age of the case group and controls were comparable. BMI, Age, gestational age, and gravidity distribution did not show any statistically significant variations between the groups (all $p > 0.05$).

H&E

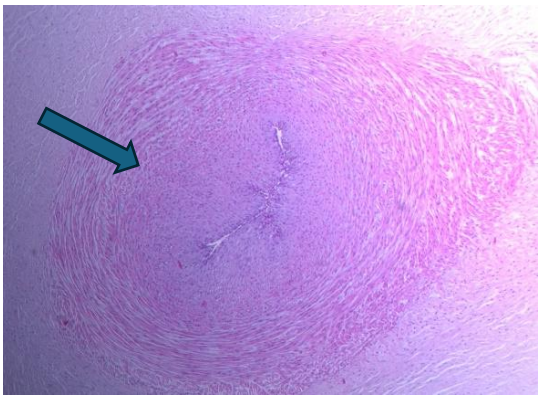

Image A

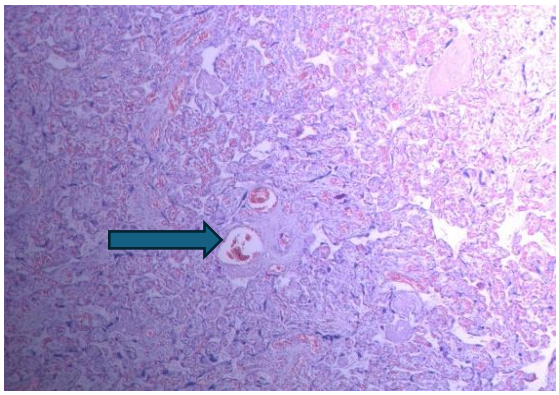

Image B

Both images are H&E-stained placental tissues. Image A (case) shows hypertrophy of the tunica media of the decidua vessel with obliterated endothelium and an almost occluded lumen. Image

B (control) shows a well-remodeled vessel with an organized endothelium and patent vessel lumen with mild hypertrophy of the tunica media.

Immunohistochemistry (IHC):

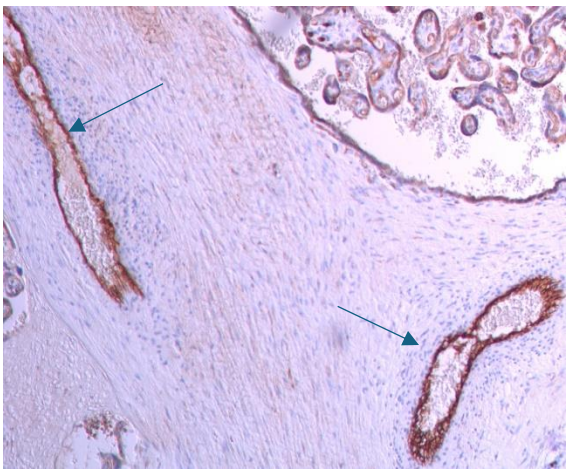

Image C

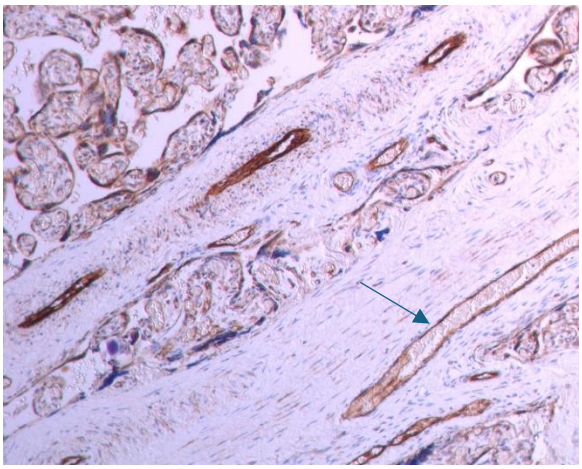

Image D

An IHC of placenta tissue from a case is shown in image C. Both arrows point to a vessel that has been intensely stained with podocalyxin. The endothelium in the vessels is aberrant and appears as spikes due to endothelial injury. Conversely, Image D shows an IHC of a control placenta, with an arrow pointing to a mildly stained vessel. The vessel is well-remodeled with a normal vessel lumen and endothelium. The image also has smaller vessels with varied levels of remodeling and staining.

Discussion

Our analysis showed no statistically significant variations in the baseline characteristics between groups. This comparison is significantly weak due to the extremely small control group (n=2). This limits the validity of concluding that the groups were well-matched. All the

participants were in their third trimester of gestation (mean gestational age 36.2 and 35.5 for cases and controls, respectively) but were not in labor at the time of sample collection.

The decidual vasculature of the case group showed hypertrophy of the vessels and obliterated endothelium. This resulted from poor remodeling of the spiral vessel, and that is known to be the basis of the pathogenesis of preeclampsia. These same changes were observed in studies conducted by Stevens et al. (Stevens et al., 2020). The poor remodeling of the vessels resulted in increased intravascular pressure, and the vessels adapted by undergoing hypertrophy to withstand the pressure. This same process led to the obliteration of the endothelial lining, which was observed as disorganized endothelial cells in the case group, as compared to the controls (Zhang, 2018). When endothelial cells break down, their constituent contents merge with the vessel's medium. Smooth muscle is destroyed, and fibrin is deposited at the same time. Placental hypoperfusion, circulating anti-angiogenic agents, and immune-mediated damage are possible triggers (Hecht et al., 2016). The control group had minor changes as a result of proper remodeling.

The immunohistochemistry showed moderate to intense staining for podocalyxin in the case group placentas. Out of the 8 placenta blocks from the case group, 5 demonstrated intense staining, and the remaining 3 had moderate staining for podocalyxin. This is due to the higher expression of podocalyxin in the spiral vessels of pregnant women with preeclampsia. The spikes seen in the endothelium infer the vascular endothelial injury in those vessels secondary to poor remodeling and increased intravascular pressure. Maternal endothelial cells are most likely the source of podocalyxin. In contrast to this study, which showed differences in immunohistochemistry staining between the case group and the control placenta, a study by Chen et al. showed that in the placenta, podocalyxin was expressed by blood vessels but not

trophoblasts, and there was no difference in its expression between preeclampsia and controls. (Chen et al., 2017).

Limitations of the study: There were several limitations of the study. To begin with, the sample size was small (18 for cases and 2 for controls). This lowered the validity of conclusions about differences between cases and controls and significantly reduced the ability to account for possible confounders. Choosing the control group presented another difficulty. It was quite challenging to find normal pregnant women without the comorbidities specified as the exclusion criteria because both cases and controls were recruited from the obstetric pathology unit, hence the smaller number of controls. We were able to obtain only 8 placentas from the 18 (39% of cases) and 1 placenta from the 2 controls, which further limited the amount of information that could be used to compare the intensity of the immunohistochemistry staining in the placenta tissues with the amount of podocalyxin in the urine. Moreover, every participant involved was in the third trimester of gestation. To determine whether podocalyxin could be used as a screening biomarker for decidual vasculopathy in preeclampsia, there should be a comparison of its concentration across trimesters.

Conclusion: These findings indicate that poor vascular remodeling in the case group is accompanied by a marked upregulation of podocalyxin. This implies that the expression of podocalyxin in the decidual vasculature of poorly remodeled vessels is higher than that of well-remodeled vessels and could serve as a biomarker for decidual vasculopathy.

Recommendations: We advise that a larger sample size for both the case and control groups be taken into account in future research. Participants from all gestational trimesters should be included in future research. Participants should be recruited from smaller clinics in addition to tertiary care facilities, as in our study, to guarantee the recruitment of patients with mild

preeclampsia who are mostly managed in community settings. Lastly, future studies should implement measures to guarantee appropriate participant follow-up for sufficient placenta retrieval following delivery.

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