Superb micro-vascular imaging of placental microcirculation in pregnant women with preeclampsia

Dai Huang
Hebei General Hospital
Dongxia Liu
Hebei General Hospital
Ying Jin
Hebei General Hospital
Li Sun
Hebei General Hospital
Lina Chen
Hebei General Hospital
Ningning Li
Hebei General Hospital
Hongyuan Xue
Hebei General Hospital
Yuquan Ye
Hebei General Hospital
Li Wang (aazhouyiyi@126.com)
Hebei General Hospital

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Abstract

Background: The purpose of this study was to explore the association of placental microcirculation with the risk of preeclampsia using superb micro-vascular imaging (SMI).

Methods: A case-control study was conducted in 2017 with 38 healthy pregnant women and 37 women with preeclampsia from the Department of Obstetrics and Gynaecology of Hebei General Hospital. The vessel counts per unit area (n/cm^2) and the hemodynamic parameters of the central part of the placenta microartery, including the pulsatility index (PI) and resistance index (RI), peak systolic velocity and diastolic velocity ratio (S/D), and time average velocity (TAV), were evaluated using superb micro-vascular imaging (SMI). The expression of CD34 in the placental tissue and the microvessel density were also calculated.

Results: The mean ages of women in the case and control groups were 31.43 and 31.24 years, respectively. Crude analyses indicated that women with preeclampsia were associated with a lower TAV ($p<0.001$) and vessel counts per unit area ($p<0.001$), whereas no significant differences were found between groups for PI ($p=0.849$), RI ($p=0.069$), and S/D ($p=0.129$) under SMI. Multiple logistic regression analysis indicated that RI (OR: 309.94; 95%CI: 2.97-32369.62; $p=0.016$), TAV (OR: 0.51; 95%CI: 0.36-0.71; $p<0.001$), and vessel counts per unit area (OR: 0.01; 95%CI: 0.00-0.86; $p=0.042$) under SMI were associated with the risk of preeclampsia, whereas PI (OR: 0.05; 95%CI: 0.00-1.34; $p=0.074$) and S/D (OR: 1.08; 95%CI: 0.39-2.96; $p=0.885$) under SMI were not associated with the risk of preeclampsia. Finally, the microvessel density of placental tissue in the preeclampsia group was significantly lower than that in the control group ($p<0.001$).

Conclusions: The findings of this study indicate that RI, TAV, and vessel counts per unit area under SMI could reflect placental microcirculation in pregnant women with preeclampsia.

Background

Preeclampsia (PE), characterized by hypertension, oedema, and proteinuria, is the most common complication encountered in obstetrics with an incidence rate of 7%-12%[1]. Serious cases of PE might have harmful effects on the health of the mother and child. The pathophysiological changes are systemic, such as small vasospasms, endothelial damage, and ischemia. The placental circulation reflects foetal blood supply and disease severity. Furthermore, the obstacles to uteroplacental microcirculation and placental blood flow reduction could cause PE, foetal growth restriction, foetal distress, and other pathological conditions associated with pregnancy[2 – 3].Therefore, the evaluation of the placental microcirculation in pregnant women with PE is of vital importance.

Superb micro-vascular imaging (SMI) is a new blood flow imaging technique that employs a unique algorithm to minimize motion artefacts by eliminating signals based on the analysis of tissue movement[4]. SMI is widely used to determine microvascular blood flow in tissue such as the breasts and

glands [5–7]. SMI has the advantage of detecting low-speed blood flow, and thus it is able to directly analyse the state of placental microcirculation in an easy and non-invasive manner, compared with other ultrasound technologies. However, no current studies have focused on SMI for placental microvascular evaluation in pregnant women during the third trimester. The CD34 antigen can label vascular endothelial cells, which is known to be superior to other markers in showing vascular endothelial cell specificity. Studies have shown that the CD34 antigen is mainly expressed in multi-functional hematopoietic stem cells, committed progenitor cells, and endothelial cells of blood vessels, making it a reliable marker for endothelial cell proliferation during angiogenesis[8]. In this study, the microvessel density (MVD) of placental tissue was counted by analysing the expression of CD34 in placental tissue, and the MVD of placental tissue was used to evaluate the distribution of microvessels to assess the severity of PE.

We conducted a case-control study to evaluate placental microcirculation using SMI on the risk of PE in pregnant women during the third trimester. Moreover, the MVD in placental tissue was compared in women with PE and healthy pregnant women using immunohistochemistry (IHC) and immunofluorescence (IF).

Methods

Patients

Thirty-seven women with single-foetus pregnancy and PE admitted to the Department of Obstetrics and Gynaecology of Hebei General Hospital between January 2017 and June 2017 were selected as the PE group. A total of 38 women with single-foetus pregnancy in their third trimester in the same hospital during the same period were selected as the control group. The diagnostic criteria for PE were based on the 2015 Chinese Medical Association Guidelines for the diagnosis and treatment of hypertensive disorders in pregnancy: systolic blood pressure≥140 mmHg and/or diastolic blood pressure≥90 mmHg, accompanied by proteinuria or associated with important organ diseases such as heart, lung, liver, and kidney after 20 weeks of gestation. The mean age and gestational age in the case group were 31.43 years and 34.52 weeks, respectively, while the mean age and gestational age in the control group were 31.24 years and 34.71 weeks, respectively. All pregnant women with other gestational complications were excluded. Only cases in which the umbilical cord was inserted into the central part of the placenta were included, and those with marginal umbilical cord insertion or sail-shaped placentas were excluded. The study was carried out following approval from the institutional review board of Hebei General Hospital, and the informed consent forms were signed by all participants.

Ultrasonographic examination

SMI was performed with a curved transducer (6C1 Aplio500; Toshiba Medical Systems Corporation, Tochigi, Japan). If the foetus was particularly quiet, the patient was asked to lie in the supine position. If the image was shaking, patients were asked to hold their breath during image acquisition. B-mode ultrasound was performed first to scan the placenta. Once detected, the ultrasound mode was switched

to the SMI mode, and the default initial sampling frame size was selected while ensuring that the placental tissue was in the frame. The settings for SMI were: colour velocity scale adjusted to 1.0–2.0 cm/s and frame rates >50 Hz. Gain settings were optimized for each round of imaging. The central vascular placenta prevail was required to be clear before the image was savedand the sampling frame area recorded (cm^2).The number of tubes in the blood sampling frame were counted by 2 physicians (with >5 years of experience in obstetric ultrasound, and 12 months of experience in SMI). During the counting process, the 2physicians were blinded to the data obtained by each other. The vasculature was counted by disregarding vasculature at the edge of the placenta close to the mother as well as large vessels at the umbilical artery insertion site close to the foetus, and counting the vasculature of the placenta that was centred in the sampling box. Branched vascular trunks were counted as separate branches, and branched vasculature and microvasculature were counted separately. The physicians counted the vasculature and the average was taken (n); the vessel counts per unit area (n/cm^2) were calculated. Positioning and detecting minimal artery in the centre of the placenta and the detection angle between the ultrasound beam and blood flow, between 0-60°, freeze frames after obtaining 3 to 5 clear and stable Doppler spectra, calculating the pulsatility index (PI) measured by automatic instrument software, resistance index (RI), peak systolic velocity and diastolic velocity ratio (S/D), and time average velocity (TAV).

Microvascular density (MVD) pathology

Immediately after delivery of the placenta, the 2 groups of pregnant women were selected from the placenta of the placenta and without significant calcification. The whole placenta was cut into 2 cm × 2 cm × 2 cm sections. The blood was replaced with normal saline, and the volume fraction was 10%. The tissue was fixed in formaldehyde solution for 12–24 h, and then stored in a refrigerator at 4 °C after dehydration, transparency, and embedding.

An antigen-antibody binding method was used for vessel detection. The antigen in this experiment was the CD34 antigen in human placental tissue, the primary antibody was a CD34 rabbit anti-human monoclonal antibody, and the secondary antibody was a biotin-labelled goat anti-rabbit secondary antibody working fluid or the fluorescent pigment FITC. Immunohistochemistry made use of a biotin-labelled secondary antibody that binds to the horseradish-labelled streptavidin working fluid to form an antigen-primary-secondary-horseradish-labelled streptavidin complex. Then, a diaminobenzidine (DAB) developer was added, which catalyses the DAB substrate and reacts at the position of the recognized antigen to form a brownish yellow precipitate, thereby showing the placental tissue. Immunofluorescence was due to a FITC label on the CD34 antigen. After binding to the CD34 antigen, a specific yellow-green fluorescence reaction occurs at the position of the antigen, thereby showing blood vessels in the placental tissue.

The expression of CD34 in the PE group and the control group was observed under a light microscope with 100-fold and 200-fold visual fields, respectively. The endothelial cells of blood vessels were labelled with the CD34 antigen and therefore were stained brownish yellow. As a result, the single stained

endothelial cells or clustered vascular endothelial cells were notably different in colour from the background. If the lumen diameter was≤100μm, the blood vessels were considered to be microvessels, and the absence of the vascular lumen was not a necessary condition for counting microvessels. The branched blood vessels were considered to be separate microvessels as long as they were not connected to each other, and they were counted according to the method described by Weidner[9] et al. The counting method was: firstly, the blood vessels positive for CD34were observed under the (×100) field of view, and the dense areas of microvessels in the tissues were determined. Then, five non-repetitive fields were selected to count in the field of (×200). The counted microvessels were averaged to determine MVD.

Statistical analysis

Continuous data between groups are shown as mean (standard deviation) and median (interquartile) based on the distribution of data, while categorical data are presented as number and frequency. The comparison between groups was performed by 2 independent sample t-tests if the data were normally distributed, while the rank sum test was employed for data with non-normal distribution. Moreover, the Chi-square and CMH methods were employed to compare the categorical data between groups according to the variables distribution met ordinal or not. Multiple logistic regression was employed to assess the potential associations of PI, RI, S/D, TAV, and the vessel counts per unit area were calculated using SMI in pregnant women with the risk of PE. All reported p-values are 2-sided, and a p-value of<0.05 was considered statistically significant. All the statistical analyses were carried out using IBM SPSS Statistics for Windows, version 19.0.

Results

A total of 37 pregnant women with PE, and 38 healthy pregnant women during the third trimester were recruited for this study. The baseline characteristics of included participants are shown in Table 1. Overall, we noted no significant differences between the case and control groups for mean age ($p=0.851$), number of pregnancies ($p=0.785$), number of labours ($p=0.602$), history of abnormal pregnancies ($p=0.143$), alcohol intake ($p=1.000$), sleep time ($p=0.240$), family history of hypertension ($p=0.571$), and gestational week ($p=0.798$). However, women in the case group had higher systolic blood pressure ($p<0.001$) and diastolic blood pressure ($p<0.001$) than those in the control group, while the distribution of salt intake between groups was statistically significant ($p<0.001$). Moreover, the birth weight ($p<0.001$) and gestational week of birth ($p<0.001$) were significantly lower in the case group.

The placental microcirculation indices under SMI between the case and control groups are shown in Table 2. Figure 1 shows the display of the central vessels of the placenta under SM in PE and healthy pregnant women at 34 weeks of gestation. The levels of TAV (10.90 vs. 14.60; $p<0.001$) and vessel counts per unit area (0.18 vs. 0.28; $p<0.001$) in the case group were significantly lower than those in the control group. Moreover, there were no significant differences between the case and control groups for PI (0.82 vs 0.76; $p=0.849$), RI (0.70 vs 0.63; $p=0.069$), and S/D (2.34 vs 2.30; $p=0.129$) under SMI. After baseline characteristics of participants were adjusted, we noted that RI (OR: 309.94; 95%CI: 2.97-

32369.62; p=0.016), TAV (OR: 0.51; 95%CI: 0.36-0.71; p<0.001), and vessel counts per unit area (OR: 0.01; 95%CI: 0.00-0.86; p=0.042) under SMI significantly correlated with the risk of PE in pregnant women during the third trimester. However, PI (OR: 0.05; 95%CI: 0.00-1.34; p=0.074) and S/D (OR: 1.08; 95%CI: 0.39-2.96; p=0.885) under SMI were not associated with the risk of PE (Table 3).

In placental tissue, CD34 is expressed in the endothelial cells of blood vessels. It was found that the vascular endothelium of the placental tissue was stained with brown-yellow particles, which could also be oblate or strip-like, and some form the lumen of blood vessels. The MVD of the placental tissue in the PE group was (104.13±10.008)/field of view, and the MVD of the placental tissue of the control group was (116.44±8.013)/field, and a significant difference was found between the groups (p<0.001; Figure 2). Figure 2A shows the expression of CD34as detected by immunohistochemistry in the placenta of the control group. The brownish-yellow part is the displayed blood vessel. The blood vessel count of the blood vessel diameter less than 100 μm is microvessel by microscopic measurement. The microvascular tube diameter was small and dense, and the distribution of the placental tissue was also dense. Figure 2B shows the expression of CD34 as detected by immunohistochemistry in the placental tissue of the PE group, which is significantly larger and sparse compared with that seen in Figure 2A. Statistical analysis revealed that the density of the placental microvessels in the PE group was significantly lower than that in the control group.

Discussion

PE in pregnant women has a harmful effect on both the mother and child and currently lacks an effective treatment strategy. Despite the aetiology and pathogenesis not being fully understood, studies have shown that the disease originates in the placenta and is related to insufficient trophoblastic infiltration and spiral artery recasting disorders[10]. The manifestations of the disease in women with severe PE include increased blood pressure and urinary protein levels, impaired maternal organ function, and placental-foetal complications[11-13]. The current case-control study analysed the associations of placental microcirculation under SMI with the risk of PE among pregnant women during the third trimester. The results of this study indicated that RI, TAV, and vessel counts per unit area under SMI were significantly associated with the risk of PE. Moreover, a significant difference in the MVD of placental tissue between groups was observed.

The results of the study showed that the number of blood vessels per unit area (n/cm^2) and TAV levels were significantly reduced in the PE group as compared with the control group. There were no significant differences in PI, RI, and S/D in the hemodynamic resistance indices. However, the PI, RI, and S/D of the PE group had an increasing trend compared with the control group. The number of blood vessels per unit area of the placenta (n/cm^2) reflects changes in placental vascular density. PI and RI are indicators of vascular compliance and elasticity, and together with the S/D values they reflect the magnitude of blood flow resistance. Higher levels of PI and RI indicated worse vascular compliance and greater vascular resistance, respectively; the higher S/D value indicated greater blood flow resistance, and TAV levels reflected the blood flow rate. All of these parameters reflected changes in the placental microcirculation.

The potential reason for the differences between groups could be that patients with PE have a series of pathological changes that may result in increased circulation resistance of the placenta[15]. These include intimal fibrosis of the small arteries of the placental bed, stenosis of the lumen, oedema of the placental chorion, degeneration, and calcification of the villi, all of which could cause the reduction of placental small arteries and placental villus blood vessels, obstruction, and decreased compliance[14]. Moreover, the reduced vascular density of the placenta, increased circulation resistance, and the reduced blood flow velocity are indicators of poor placental perfusion in pregnant women with PE.

In this study, the MVD of placental tissue was used to evaluate the distribution of microvessels, and the severity of PE[16,17]. Moreover, immunohistochemistry and immunofluorescence were used to detect the MVD of placental tissue in this study. The results showed that the microvascular vessels positive for CD34 in the placenta of the control group were small and dense, and the distribution of placental villus tissue was dense. The microvascular diameter of the placental tissue in the PE group was significantly increased, and the MVD was significantly reduced. That is, the microvessels of the placental tissue of the PE group were significantly increased and sparse compared with those of the control group, which may be related to PE. The wall of the spiral arteriole of the placenta is thickened, and atherosclerosis occurs, which leads to obstruction of the lumen, which in turn leads to chorionic ischemic infarction. Further explanation of the existence of placental microcirculation in patients with PE could be that formation and proliferation of placental blood vessels is inhibited to varying degrees and blood perfusion is insufficient, causing ischemia and hypoxia of the placenta causing development of the vascular network of the placenta and remodelling dysfunction of the placental vascular bed, which may be the main cause of PE.

Several shortcomings of this study should be addressed. First, the current study was designed as a case-control study and all women were from a single hospital, which might have induced potential confounder biases including selection and recall bias. Second, stratified analyses based on the characteristics of women were not conducted due to the small number of included individuals. Third, the mechanism of reducing the MVD of placental tissue in patients with PE was not analysed at the molecular level, which should be done in future studies. Finally, the characteristics of placenta development such as placental weight were not recorded or statistically analysed in this study.

Conclusions

In conclusion, there were significant differences between the PE and control groups for RI, TAV, and vessel counts per unit area under SMI. Moreover, the MVD of placental tissue in the PE group was lower than that in healthy women. Further prospective and experimental studies should be conducted to verify the results of this study and explore the mechanisms of MVD in patients with PE at a molecular level.

Abbreviations

PI: pulsatility index; RI: resistance index; S/D: peak systolic velocity and diastolic velocity ratio; TAV: time average velocity; SMI: superb micro-vascular imaging; PE: preeclampsia; MVD: microvessel density; IHC:

immunohistochemistry; IF: immunofluorescence; DAB: diaminobenzidine; OR:odds ratio; CI: confidence interval

Declarations

Authors' contributions

Study design and manuscript drafted: HD, YY, and WL; Data collection and interpretation: LD, JY, SL, CL, LN, XH; Data analysis: HD and LD; manuscript revised: YY and WL. All authors have approved the submitted version, have agreed both to be personally accountable for the author's own contributions and to ensure that questions related to the accuracy or integrity of any part of the work. The correspondence authors attest that all listed authors meet authorship criteria.

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Availability of data and materials: The data of this study are stored in secured servers of the institutions and are available from the corresponding authors on reasonable request.

Ethics approval and consent to participate: The study was carried out following approval from the institutional review board of Hebei General Hospital, and the informed consent forms were signed by all participants.

Consent for publication: Not applicable

Competing interests: The authors declare that they have no competing interests.

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Tables

Table 1. Baseline characteristics of pregnant women in preeclampsia and control group

Variable	Control (n=38)	Case (n=37)	Statistic	P value
Mean age	31.24(4.41)	31.43(4.59)	0.19	0.851
Number of pregnancy				
1	4(10.53)	7(18.92)	0.07	0.785
2	16(42.11)	11(29.73)		
3	10(26.32)	12(32.43)		
4	7(18.42)	6(16.22)		
5	1(2.63)	1(2.70)		
Parity				
0	10(26.32)	12(32.43)	0.27	0.602
1	27(71.05)	24(64.86)		
2	1(2.63)	1(2.70)		
Abnormal pregnancy history				
None	17(44.74)	26(70.27)	5.42	0.143
Ectopic pregnancy	1(2.63)	0(0.00)		
Embryo damage	4(10.53)	2(5.41)		
Fetal malformation	0(0.00)	0(0.00)		
Stillbirth	0(0.00)	0(0.00)		
Other	16(42.11)	9(24.32)		
Diagnosis of gestational week	-	30.03(4.96)	-	-
SBP (mmHg)	104.00 (100.00,105.00)	164.00 (160.00,180.00)	7.45	<0.001
DBP (mmHg)	72.00 (71.00,76.00)	110.00 (107.00,115.00)	7.47	<0.001
Urine protein (positive)	-	30(81.08)	-	-
Alcohol (never)	37(97.37)	37(100.00)	-	1.000
Salt habit				
Light	6(15.79)	20(54.05)	14.03	<0.001
Moderate	28(73.68)	17(45.95)		
Heavy	4(10.53)	0(0.00)		
Sleep time (hours)				
6-8	14(36.84)	9(24.32)	1.38	0.240
> 8	24(63.16)	28(75.68)		
Family history of hypertension				
No	23(60.53)	20(54.05)	0.32	0.571
Yes	15(39.47)	17(45.95)		
Gestational week	34.71(2.94)	34.52(3.50)	0.26	0.798
Birth weight (Kg)	3.75 (3.20,3.85)	2.10 (1.50,2.70)	5.42	<0.001
Gestational week of birth	39.30 (39.00,39.60)	34.60 (33.30,38.60)	5.34	<0.001

Table 2. Placental microcirculation indexes under superb micro-vascular imaging mode between preeclampsia and control group

Parameters	Control (n=38)	Case (n=37)	Statistic	P value
PI	0.76 (0.65,0.93)	0.82 (0.67,0.92)	0.19	0.849
RI	0.63 (0.50,0.89)	0.70 (0.65,0.90)	1.82	0.069
S/D	2.30 (1.98,2.54)	2.34 (2.23,2.52)	1.52	0.129
TAV (cm/s)	14.60 (12.50,16.80)	10.90 (9.50,11.50)	5.19	<0.001
Count per unit area of vessel counts(cm^2)	0.28 (0.22,0.35)	0.18 (0.14,0.24)	3.45	<0.001

Table 3. Multiple logistic regression analysis for placental microcirculation indexes under superb micro-vascular imaging mode

Parameters	β	Standard error	Wald Chi-square	OR (95% CI)	P value
Intercept	7.582	2.586	8.598	-	0.003
PI	-3.036	1.697	3.202	0.05(0.00-1.34)	0.074
RI	5.736	2.372	5.850	309.94(2.97-32369.62)	0.016
S/D	0.075	0.516	0.021	1.08(0.39-2.96)	0.885
TAV (cm/s)	-0.683	0.172	15.688	0.51(0.36-0.71)	<0.001
Count per unit area of vessel counts(cm^2)	-4.236	2.082	4.137	0.01(0.00-0.86)	0.042

Figures

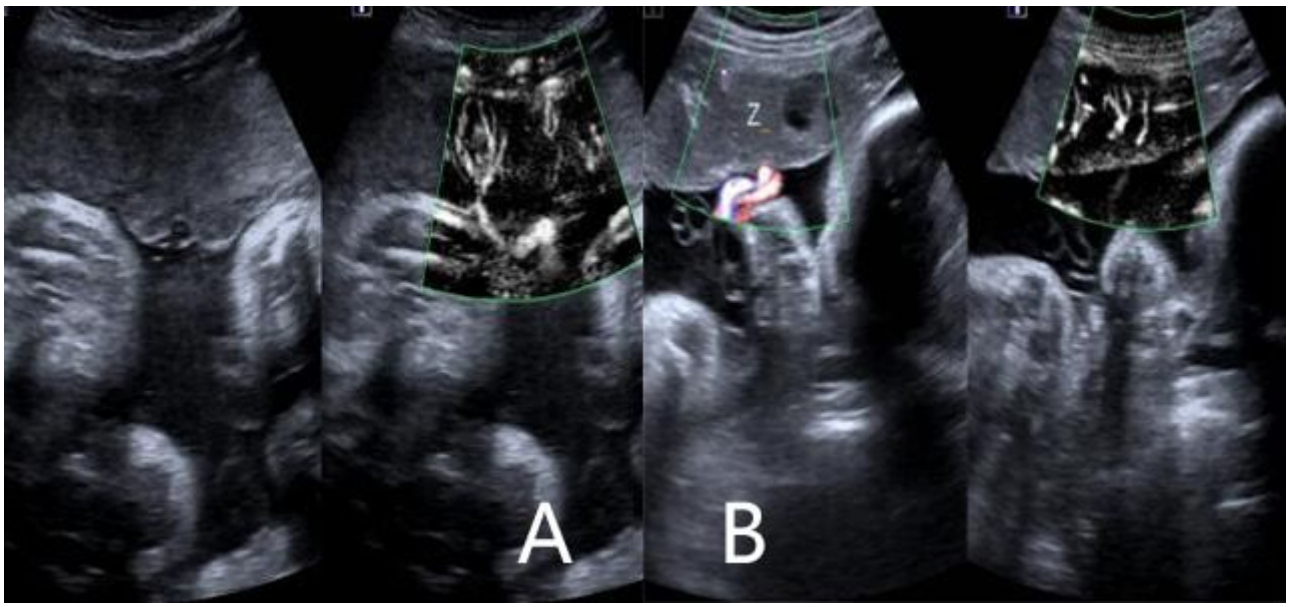

Figure 1

Note A:The display of the central vessels of the placenta under SMI model of preeclampsia at 34 weeks of gestation. Note B：The display of the central vessels of the placenta under SMI model of healthy pregnant women at 34 weeks of gestation.

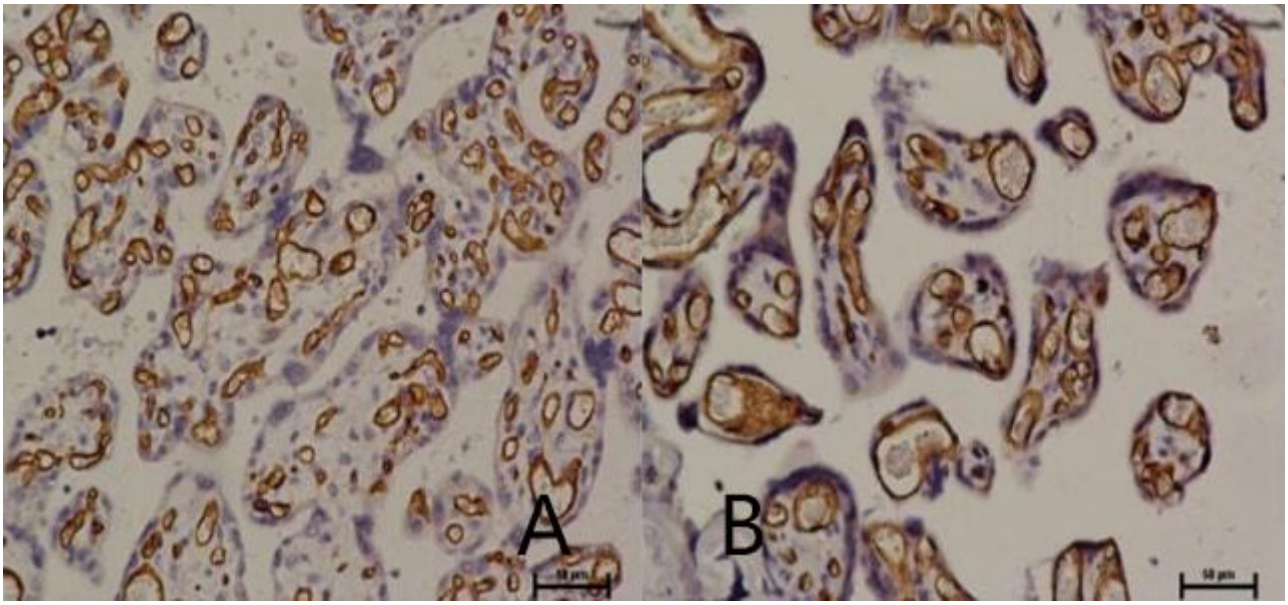

Figure 2

Immunohistochemistry and immunofluorescence images expressed MVD. Note A: Control group; Note B: preeclampsia group