Vascular corrosion casting of normal and pre-eclamptic placentas

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Abstract. Pre-eclampsia is an important cause of maternal and fetal morbidity and mortality that is associated with decreased placental perfusion. In the present study, vascular corrosion casting was used to investigate the differences in structural changes of the fetoplacental vasculature between normal and pre-eclamptic placentas. An improved epoxy resin vascular casting technique was used in the present study. Casting media were infused into 40 normal and 40 pre-eclamptic placentas through umbilical arteries and veins in order to construct three dimensional fetoplacental vasculatures. The number of branches, diameter, morphology and peripheral artery-to-vein ratio were measured for each specimen. The results indicated that the venous system of normal placentas was divided into 5-7 grades of branches and the volume of the vascular bed was 155.5±45.3 ml. In severe pre-eclamptic placentas, the volume was 106.4±36.1 ml, which was significantly lower compared with normal placentas (P<0.01). The venous system of pre-eclamptic placentas was divided into 4-5 grades of branches, which was much more sparse compared with normal placentas. In additions, the diameters of grade 1-3 veins and grade 2-3 arteries were significantly smaller in severe pre-eclampsia (P<0.05). In conclusion, pre-eclamptic placentas displayed a decreased volume of vascular bed, smaller diameters of grade 1-3 veins and grade 2-3 arteries, and an increased peripheral artery-to-vein ratio, which may be a cause of the placental dysfunction during severe pre-eclampsia.

Introduction

Pre-eclampsia, a pregnancy-specific disorder characterized by hypertension and proteinuria after the 20th week of pregnancy, is a major cause of maternal and fetal morbidity and mortality worldwide (1). The exact etiology of pre-eclampsia is unclear. It is considered to be a multifactorial disease caused by complex interactions between genetic and environmental factors affecting the immune and vascular functions (2). Early diagnosis is critical for improving the placental function, preventing life-threatening complications, and reducing maternal and fetal mortality. Despite the multifactorial characteristics of pre-eclampsia, the majority of cases are associated with abnormal maternal uterine vascular remodeling by variant placental trophoblastic cells (2,3). Placental abnormalities, including alterations in the uteroplacental vasculature, have been identified in pre-eclampsia research (4-7). Therefore, it has been hypothesized that there are differences in the structural changes occurring in the fetoplacental vasculature between normal and pre-eclamptic placentas, and it was these differences that were investigated in the current study.

Vascular casting is an effective way of investigating various diseases (8,9). A number of researchers have used computed tomography scanning or scanning electron microscopy to examine the three-dimensional structure of blood vessels (10-12), while the vascular corrosion casting technique has been used to reconstruct the vasculature (13). The vascular corrosion casting technique produces a replica of the vascular beds by injecting a plastic polymer that fills the blood vessels and rapidly polymerizes. Surrounding tissues are subsequently corroded away with alkali solutions. Following washing and drying, the hardened cast representing the geometry of the original vascular system can be examined (14).

To the best of our knowledge, there have been no studies using the vascular corrosion casting technique to examine and compare the uteroplacental vasculatures in placentas of normal term pregnancy and pre-eclampsia pregnancy. Therefore, the experiments in the current study were performed to establish the vascular corrosion casting of normal and pre-eclampsia placentas.

Materials and methods

Patients and materials. A total of 80 placentas from 80 female patients aged 26-37 years old were randomly collected in the Department of Obstetrics and Gynecology at the Jinan Military General Hospital (Jinan, China) between October 2007 and October 2010. Informed consent was obtained from each patient included in the current study. Among these, 40 samples were obtained from normal term pregnancies with an average gestational age of 39.5±2.4 weeks from patients with an average age of 28.5±2.5 years, while 40 were from pre-eclampsia cases from patients with an average age of 27.0±3.5 years (26 mild

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cases with an average gestational age of 38.5±2.5 weeks, and 14 severe cases of 33.0±2.0 weeks). Among the 40 patients with pre-eclampsia, 29 patients underwent vaginal delivery and 11 underwent cesarean section. Among the 40 patients that experienced a normal term pregnancy, 32 ones underwent vaginal delivery and 8 underwent cesarean section. The criteria for pre-eclampsia diagnosis were based on the 23rd edition of Pregnancy Hypertension in Williams Obstetrics (15). The present study was approved by the Institutional Ethics Committee of Jinan Military General Hospital.

Epoxy resin (Jinan resin composite materials plant co., Ltd., Jinan, China) was used for uterovascular casting. The blood vessels of each placenta were infused with a mixture of the following vascular casting materials: 300 ml epoxy resin, 40 ml dibutyl phthalate (Shandong Yuanli Science and Technology Co., Ltd, Weifang, China), 40 ml acetone, 40 ml ethylenediamine (HaoRui chemical (Shanghai) Co., Ltd., Shanghai, China), 3 ml red oil paint and 3 ml blue oil paint (Both Yancheng City Everbright Pigment co., Ltd., Jiangsu, China). More specifically, in order to make the casting media, acetone (used as a thinner) and dibutyl phthalate (used as a plasticizer) were added to epoxy resin, and then ethylenediamine (used as a fixative) was added. Subsequent to mixing by stirring, the mixture was divided into two parts, and red or blue paint was added.

Two types of self-designed spiral metal pressurizing units, which could be selected according to the perfusion volume required, were used along with 20 or 50 ml disposable plastic syringes (Fig. 1) for injecting the casting media into the placental vessels. This procedure involved a perfusion process of the mixture of the vascular casting materials, as greater perfusion material viscosity requires a large thrust.

Processing of placentas. Subsequent to delivery, the placentas were immediately bathed in 500 ml normal saline containing 100 IU heparin and blood clots were removed. Cannulas were inserted into the umbilical vein and two umbilical arteries, and secured. The blood vessels were washed with normal saline containing heparin until the placentas became pale. Next, 50 ml syringes filled with casting media were connected to the pressurizing unit. The red-dyed casting media were first injected into the umbilical veins, and then the blue-dyed casting media were injected into the umbilical arteries. The perfusion speed was 1.0 ml per 5 sec. Once the colored casting exuded profusely from the placental chorion surface, indicating that enough had been injected to the umbilical arteries, the cannulas were removed and the umbilical veins and arteries were ligated. The placentas were kept in clean water overnight to allow the casting media to solidify. Once hardened, specimens were soaked in 30% copper hydroxide to corrode away the placental tissues. The specimens were rinsed with water once every 3 days. Finally, the placental vascular casts were stored in water in specimen containers.

A water displacement method was used to measure the volume of the placental vascular bed. Briefly, the specimen was inserted into a graduated cylinder with appropriate amount of water that was sufficient to cover it, and the volume was read. The difference between the original and final volumes was recorded as the placental vascular volume. To calculate the artery-to-vein ratio per cm^2 and examine the peripheral vascular network, 10 spots were selected along the edge of the placentas, and a further 10 spots were selected in between the umbilical cord and the edge of the placenta. The levels of branching and the diameters of placental veins and arteries were also recorded.

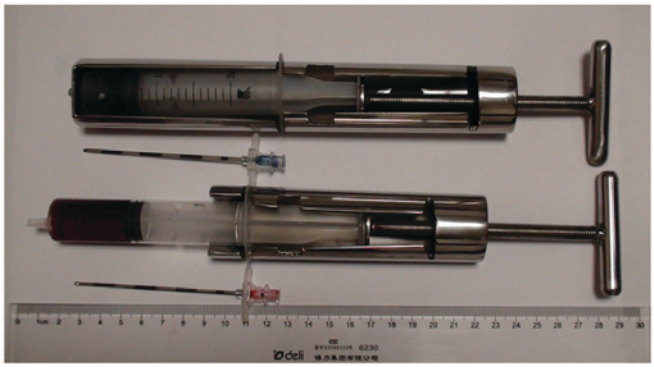

Figure 1. Images of the two types of self-designed spiral metal pressurizing units used along with 20 or 50 ml disposable plastic syringes and puncture needles for injecting the casting media into placental vessels.

Statistical analysis. SPSS version 13.0 (SPSS Inc., Chicago, IL, USA) was used for all statistical analyses. Student's *t* test was used to compare the placental vascular volumes, branching levels and the diameters of various grades of blood vessels between the normal and pre-eclamptic placentas. $P<0.05$ was considered to indicate statistically significant differences between the placentas.

Results

Features of normal placental vasculature. The volume of normal placental vascular bed was 155.5±45.3 ml, while the peripheral artery-to-vein ratio ranged between 1:2 and 1:3. The results of the perfusion study demonstrated that the placental venous system was divided into 5-7 grades (Table I). To the best of our knowledge, this branch system has not previously been described. For specific performance, the umbilical vein, which was defined as a grade 1 vein of placental vessels, gradually branched into grade 2 to grade 7 veins upon entering the placenta, tapering uniformly towards the periphery and ending in an abundant placental capillary network. The umbilical vein (grade 1; located inside the umbilical cord, carrying oxygenated blood into the fetus) of normal placentas had a diameter of 8.7±2.4 mm. Grade 2 veins (branched from grade 1 vein) consisted of 3-4 veins with a diameter of 4.5±1.1 mm. Grade 3 veins (branched from grade 2 veins) had a diameter of 3.5±1.0 mm. The two umbilical arteries (grade 1; located inside the umbilical cord, carrying deoxygenated blood out of the fetus) connected with each other right before the cord insertion to form a vascular sinus. These were then gradually divided into subordinate arteries that are reduced by 1-2 levels compared with the venous system, forming a peripheral artery network that was more sparse than the peripheral venous network. The umbilical arteries (grade 1) of normal placentas had diameter of 3.6±1.4 mm. The grade 2 arteries (branched from grade 1 arteries, consisting of 3-4 branches) had a diameter

Table I. Comparison of the vascular casts of normal and pre-eclamptic placentas (mean ± standard deviation).

Group	n	Placental vascular volume (ml)	Grades of veins	Features of peripheral vascular network	Peripheral artery: vein ratio
NP	40	155.5±45.3	6.1±1.2	Dense, smooth	0.4±0.3
Mild PE	26	140.8±50.6	5.9±1.1	Dense, smooth, sparse, nodulous in certain areas	0.4±0.2
Severe PE	14	106.4±38.1[a]	4.3±1.3[b]	Sparse, nodulous	0.6±0.2[b]

[a]P<0.01, [b]P<0.05 vs. the NP group. NP, normal pregnancy; PE, pre-eclampsia.

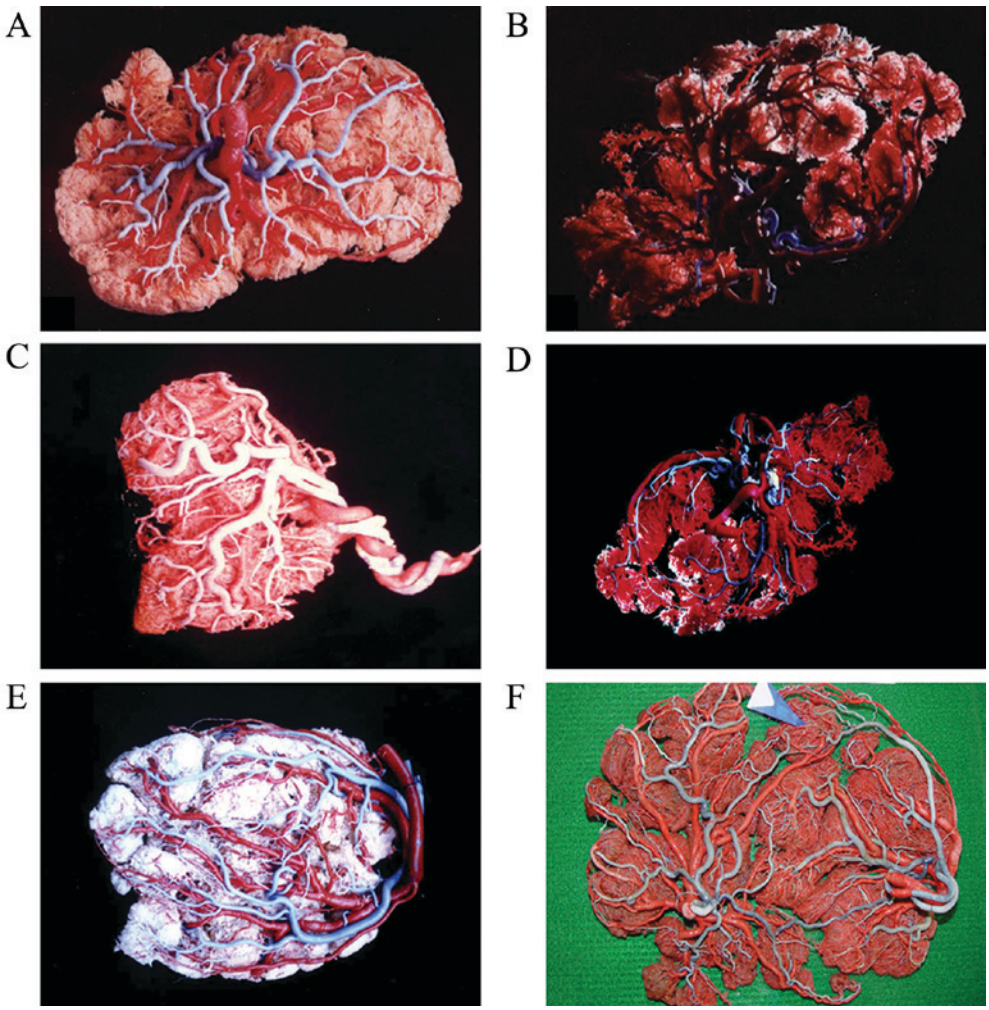

Figure 2. Images of placental vasculature casts of normal pregnancy and pre-eclampsia. (A) The amniotic and (B) chorionic sides in two different representative normal pregnancy specimens are presented. (C) The amniotic and (D) chorionic side in a representative pre-eclamptic placental sample are also presented. (E) Velamentous cord insertion, with the lower part of the umbilical cord found outside of the placenta. (F) Placenta from a dizygotic twin pregnancy, with visible lower part of the umbilical cords and microvascular anastomosis between the two placentas. In each image, the placental venous system (with oxygen blood) is shown in red, while the two umbilical arteries (anoxemia), are shown in blue.

of 3.0±1.3 mm. In addition, the grade 3 arteries of normal placentas (branched from grade 2 arteries) had a diameter of 1.7±0.5 mm. The amniotic (Fig. 2A) and chorionic sides (Fig. 2B) of placental vasculature casts from a normal pregnancy specimen are presented here.

Placental vasculature features of pre-eclampsia specimens. As presented in Table I, the vasculatures of placentas with mild pre-eclampsia (140.8±50.6 ml) did not significantly differ from those of normal placentas (155.5±45.3 ml) in terms of vascular volume (P=0.5321), levels of branching (5.9±1.1 vs. 6.1±1.2) or peripheral artery-to-vein ratio (0.4±0.2 vs. 0.4±0.3; P=0.5734). However, the vascular volume of severe pre-eclampsia placentas was 106.4±38.1 ml, which was significantly reduced compared with that of normal placentas (P=0.0251). Levels of branching (4.3±1.3 vs. 6.1±1.2; P=0.0451) and peripheral

Table II. Comparison of grades 1 to 3 of placental vessels between the normal and pre-eclamptic placentas (mean ± standard deviation).

		Vein diameter (mm)			Artery diameter (mm)		
Group	n	Grade 1	Grade 2	Grade 3	Grade 1	Grade 2	Grade 3
NP	40	8.7±2.4	4.5±1.1	1.8±0.5	3.6±1.4	3.0±1.3	1.7±0.5
Mild PE	26	8.0±1.8	4.1±1.3	1.7±0.7	3.6±1.2	2.6±1.2	1.4±0.6
Severe PE	14	5.7±1.3[a]	2.8±1.1[b]	1.1±0.3[b]	3.3±1.2	1.5±0.6[b]	0.7±0.3[b]

[a]P<0.05, [b]P<0.01 vs. the NP group. NP, normal pregnancy; PE, pre-eclampsia; Grade 1, located in the umbilical cord; Grade 2, branched from grade 1 veins or arteries (consisting of 3-4 branches); Grade 3, branched from grade 2 veins or arteries.

Sartery-to-vein ratio (0.6±0.2 vs. 0.4±0.3; P=0.0347) were also significantly reduced (Table I; Fig. 2C-F).

The diameter of grade 1 veins in pre-eclamptic placentas was 5.7±1.3 mm, while grade 2 veins consisted of 3-4 branches. The diameters of grade 1-3 veins were significantly smaller than veins of the same grade in normal placentas (P=0.0282; Table II). Grade 1 umbilical arteries had a diameter of 3.3±1.2 mm, which was not significantly different from those of normal placentas (P=0.0815; Table II). Grade 2 arteries consisted of 3-4 branches. In addition, the diameters of grade 2 and 3 arteries were significantly smaller compared with arteries of the same grade in normal placentas (P=0.0370; P<0.05, respectively; Table II). The venous system of severe pre-eclampsia placentas had 4-5 levels of branches, meaning that they were markedly more sparse compared with normal placentas. Furthermore, grade 2-4 veins demonstrated uneven diameters and an irregular surface with nodularity. Umbilical arteries consisted of 4-5 levels of branches. The number of peripheral arterial branches was relatively higher as compared with the number of peripheral vein branches, resulting in an artery-to-vein ratio of 2:3 to 1:1 (Table I).

Discussion

Pre-eclampsia is a major contributor to intrauterine growth retardation and perinatal mortality (2) Khong *et al* (16) reported that the vascular bed of pre-eclamptic placenta exhibits apparent abnormality. In pre-eclampsia, the spiral arteries undergo acute atherosis due to defective vascular remodeling, leading to ischemic-hypoxic placental vascular lesion, which in turn decreases the placental perfusion and severely affects fetal development (3,4). Peker *et al* (17) investigated the intraplacental course and morphometrics of the umbilical artery in pre-eclamptic placentas using vascular corrosion casting. The authors determined that, although there was no significant difference in the gross anatomy of the arteries compared with normal placentas, the intervillous space was expanded in pre-eclamptic placentas. Using immunohistochemistry, Starostina *et al* (6) observed that circulating immune complexes and levels of immunoglobulin A, M and G were elevated in the placental chorionic villi and spiral arteries. Meanwhile, trophoblasts in uterine veins were markedly higher compared with those in normal pregnancy. Therefore, there were increased trophoblastic antigens entering maternal blood circulation, leading to an increase in deposits of circulating immune complexes on the basement membranes. The deposition of immunocomplexes may serve an important role in the immunopathology of placental lesions. Previous studies using electronic microscopy and immunohistochemistry have indicated that pathological changes in placental vasculature may result from placental hypoxia-ischemia in pre-eclampsia (6,7).

In the present study, an improved vascular corrosion casting technique was used in order to visualize the vascular bed, as it has been determined that acute atherosis may occur in pre-eclampsia (16), thus leading to a decrease in placental vascular volume. Observation of the vascular models obtained in the current study revealed a number of facts. Firstly, the placental vascular volume in severe pre-eclampsia (106.4±38.1 ml) was significantly lower compared with normal placentas (155.5±45.3 ml), however, the vascular volumes in normal placentas compared with mild pre-eclampsia placentas did not differ significantly. In addition, the results indicated that the branching patterns of the umbilical vein and arteries differed between normal and pre-eclamptic placentas. In normal placentas, the venous system had abundant branching that was divided into 5-7 levels. The umbilical vein was the grade 1 vein, which branched into grade 2-7 veins progressively, decreasing uniformly in diameter and ending in a dense peripheral network of placental capillaries. By contrast, in the placentas of severe pre-eclampsia, the umbilical vein was divided into 4-5 levels of branches, which were markedly reduced compared with those in normal placentas. Grade 2-4 placental veins in pre-eclamptic placentas exhibited uneven diameters in certain areas and irregular surface with nodularity and depressions. The two umbilical arteries of the placentas were connected, forming a vascular sinus at the foot of the umbilical cord before branching into subordinate arteries. The branching of the umbilical arteries was also different between normal and pre-eclamptic placentas. Normal umbilical arteries had 5-6 levels of branches, forming a peripheral artery network that was more sparse than the peripheral venous network, whereas the umbilical arteries of pre-eclamptic placentas had 4-5 levels of branches.

The present study also observed that, in placentas from pregnancies complicated with severe pre-eclampsia, the peripheral artery-to-vein ratio was increased compared with that in normal placentas. In normal placentas, the peripheral

artery-to-vein ratio ranged between 1:2 and 1:3 (0.4±0.3), whereas the ratio in pre-eclamptic placentas was between 2:3 and 1:1 (0.6±0.2). Furthermore, the vessel diameters differed between normal and severe pre-eclampsia placentas. In severe pre-eclamptic placentas, the diameter of grade 1-3 veins were significantly decreased as compared with veins of the same grade in normal placentas. In addition, diameters of grade 2-3 placental arteries were significantly reduced in placentas of severe pre-eclampsia patients compared with the normal placentas. However, there was no significant difference in the features of the vascular networks (vascular volume and peripheral artery-to-vein ratio) between placentas of mild pre-eclampsia and normal placentas.

It is postulated that there are three potential reasons for the decreased vascular volume and vessel diameters, as well as the increased peripheral artery-to-vein ratio in placentas of severe pre-eclampsia. Firstly, these features may be a result of hypertension, which induces angiospasm and angiosclerosis of the placental blood vessels, with the severity increasing with the degree of hypertension. This may lead to decreased vascular volume. Another reason may be the deposition of immunocomplexes in the vessel walls of uteroplacental vasculature, leading to narrowing of blood vessels and poor blood flow. Thus, certain small blood vessels become obstructed and cause the blood flow to stop in local areas, which also leads to a decreased volume in the placental vascular bed and increased peripheral artery-to-vein ratio. Finally, these features may be a result of the pressure in the placental veins, which is higher than that of the placental arteries. Therefore, the vessels of the placental venous system are more severely damaged compared with the arteries, leading to more severe obstruction in the venous system and increased peripheral artery-to-vein ratio. This is more apparent in severe pre-eclampsia.

The vasculature casts of normal and pre-eclamptic placentas constructed in the present study demonstrated that there were morphological differences between the two groups. In placentas from normal pregnancies, the umbilical veins divided into grade 2-7 branches, tapering uniformly and ending in an abundant peripheral capillary network. This structure increases the exchange areas of the blood vessels, ensuring maximum oxygen supply to the fetus. The two umbilical arteries that carry blood into the placenta are connected to form a vascular sinus at the lower part of the umbilical cord prior to dividing into branches that are reduced by 1-2 levels compared with the veins. This facilitates the blood flow into the placenta. The peripheral arterial network is more sparse than the venous network, with a peripheral artery-to-vein ratio of 1:2 to 1:3. In severe pre-eclampsia, the placental vascular volume was reduced to 68.4% (106.4 vs. 155.5 ml) of the normal placental vascular volume. The branching of placental veins was also decreased, and the peripheral venous network was more sparse compared with normal placentas, resulting in a peripheral artery to vein ratio of 2:3 to 1:1. This indicated a decreased exchange area in the placental villi. In severe pre-eclampsia, not only the vascular volume decreased in, but the diameters of the vessels also decreased, which is another manifestation of insufficient perfusion. In severe pre-eclamptic placentas, the diameters of placental veins were reduced compared with those in normal placentas. Notably, the veins of severe pre-eclamptic placentas exhibited uneven diameters and irregular surface with nodularity and depressions, which may result from immunocomplex deposition on the vessel walls (5,6).

In conclusion, the human placental vascular casts constructed in the present study provided a valuable tool for understanding the structures of uteroplacental vasculatures. These casts may be used as models for visualizing placental structures and investigating placental functions in various diseases, such as pre-eclampsia. However, a limitation of the present study was that the differences in the volumes of the venous system and the arterial system were not analyzed separately between normal and pre-eclamptic placentas, which may have important clinical relevance.

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