CD34 expression of chorionic villous in pre-eclamptic placenta: an immunohistochemical and ultrastructural study

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Summary

In this study, pre-eclampsia, proteinuria, and edema associated with hypertension in pregnancy were assessed at the Dicle University School of Medicine Department of Obstetrics and Gynecology Clinic. One group included 20 pre-eclamptic pregnant women with gestational age 20-35 weeks of pregnancy and the same in the control group that included; however, 20 normotensive pregnant women. Histochemistry, immunohistochemistry, and electron microscopy techniques were used. Histopathological examination of syncytial nodes and stromal cells were observed in the increase in hyperplasia and hyalinization. The evaluation immunohistochemical of chorionic villi, placenta, and hematopoietic stem cell markers showed a positive reaction with CD34. Ultrastructural examination showed endoplasmic reticulum dilatation, degeneration of mitochondria in endothelial cells, and capillary vessel edema.

Key words: Pre-eclampsia; CD34; Placenta.

Introduction

Pre-eclampsia is a disorder with widespread vascular endothelial malfunction and occurs after 20 weeks gestation. It is clinically defined by hypertension and proteinuria, with or without pathologic edema. It is widely accepted that deficient trophoblast invasion of the spiral arteries leading to inadequate blood supply to the fetus is the central pathological feature [1, 2]. The development of fetal placental vasculature depends on the actions of angiogenic growth factors and their receptors produced by cells and extracellular matrix in close proximity to the fetal vessels [3]. Maternal endothelial cell dysfunction is a root cause of the peripheral vasoconstriction that characterizes pre-eclampsia and the multiorgan damage that often occurs in severe cases [4]. CD34 monocolonal antibodies are expressed selectively on human hematopoietic progenitor cells, including myeloid and lymphoid progenitors [5]. Fetal CD34+ cells enter the maternal circulation during pregnancy and may persist for decades. These cells are usually depicted as hematopoietic stem/progenitor cells [6].

CD34 expression compared with the pre-eclamptic and normotensive placentas demonstrated the effect of angiogenesis.

Materials and Methods

This study was conducted at the Dicle University Faculty of Medicine Department of Obstetrics and Gynecology clinic and 20 pre-eclamptic pregnant women with gestational age 20-35 weeks of pregnancy and the same as the control group included 20 normotensive pregnant women. The patients were classified according to diastolic blood pressure: normotensive, with diastolic blood pressure between 80 and 90 mmHg; between 90 and 100 mmHg; between 100 and 110 mmHg, or 110 mmHg and above. Immediately after delivery, normal and pathological placentas were transported from the delivery room to the laboratory and, after preliminary gross examination, two series of tissue samples were obtained. The specimens were immersed in 10% buffered formaldehyde. Then, sections of five μm in thickness were cut and made into slides. These were processed for hematoxylin and eosin (H&E) and trichrome masson staining, carried out according to conventional procedures.

Immunohistochemical technique

Immunostaining was carried out using the avidin-biotin-peroxidase complex method. After deparaffinization and dehydration, endogenous peroxidase activity was blocked using three percent hydrogen peroxide in pure methanol for ten minutes at room temperature. The tissues were then treated with 0.01% pepsin in 0.01M HCl at 37°C for 15 minutes. After serum blocking, using two percent bovine serum albumin, the sections were then incubated with the primary antibody for 30 minutes at room temperature. The primary antibody was a mouse monoclonal antibody for low molecular weight cytokeratin at a dilution of one in 40. This was then incubated using the secondary antibody CD34 for 30 minutes.

Table 1. — *Expected values, measured values, and SI values of quality control samples.*

Groups	Pre-eclampsia group (n = 20)	Control group (n = 20)	
Mean 24-hour proteinuria	1.6 (± 1.5)		
Mean diastolic blood pressure	123 (± 9.7)	92 (± 1.3)	$p < 0.001$
Mean blood pressure	106 (± 9.4)	80 (± 1.9)	$p < 0.001$
Chorion villus diameters	6,821.55 ± 24,451.84 Mean 16,300.09, Std 4,412.02	5,428.42 ± 13,086.36 Mean 8,192.06, Std 2,679.8	
Vessel diameters in chorion	Mean 950.96, Std 1,347.82 224.32 ± 4,783.02	Mean 135.32, Std 68.79 82.74 ± 330.99	

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Fig. 1a

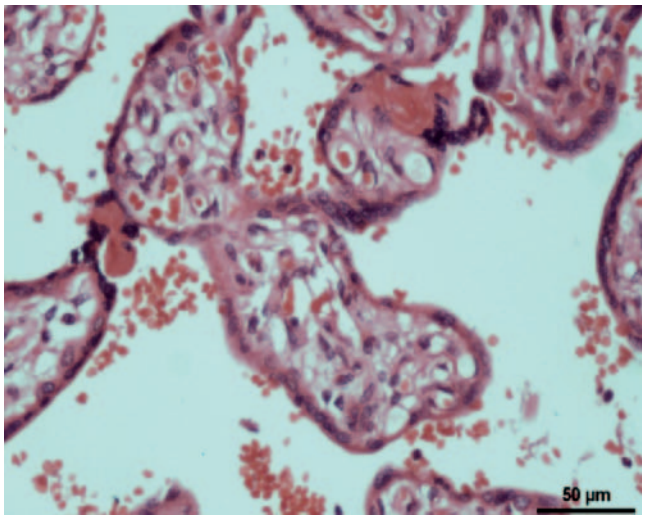


Fig. 1b

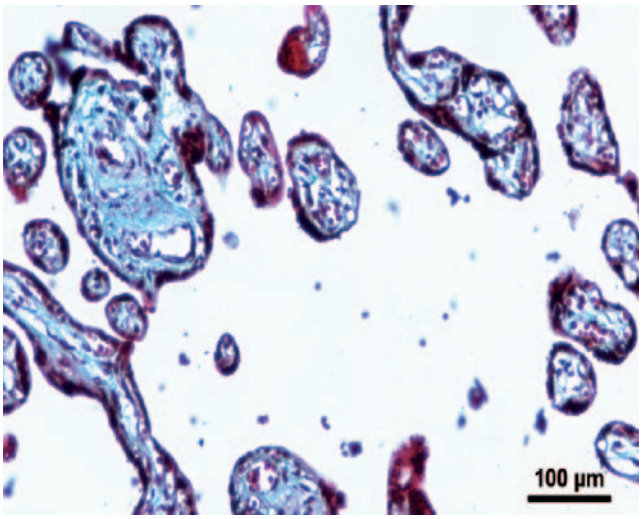


Fig. 2

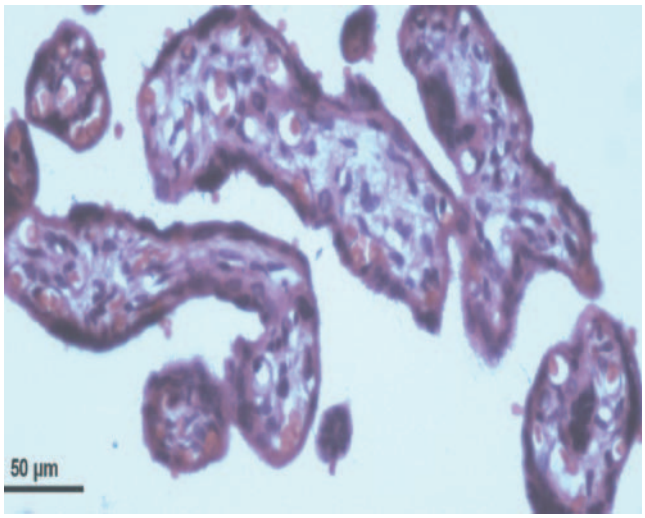


Figure 1. — Pre-eclamptic group: increased syncytial nodes, vessels, hemorrhage, and red cell distribution in a free state in the intervillous (a) (H&E stain) an increase in connective tissue and chorionic villus (b) Trichrom Masson stain.

Figure 2. — Control group, the normal appearance of chorionic villi (H&E).

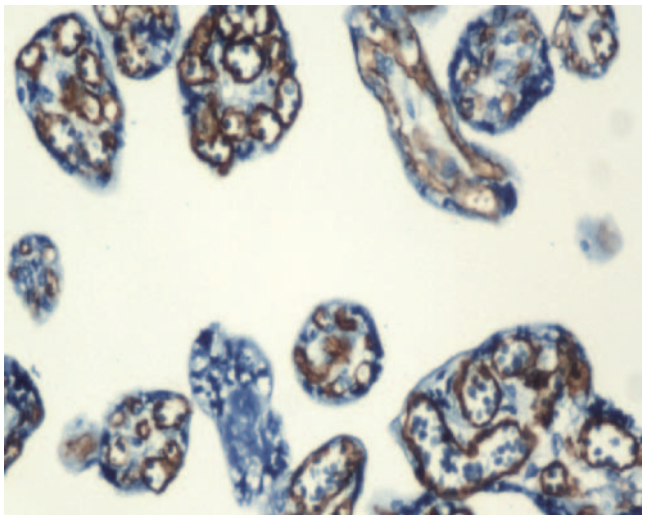
Electron microscopy technique

The pieces of tissue were immediately placed in 2.5% buffered glutaraldehyde for four hours, then fixed in OsO_4 for two hours, dehydrated in graded ethanols, and embedded in araldite. Semithin sections one μm thick were cut and stained with methylene blue-azure II for light microscopic examination. Thin 70-nm thick sections were stained with lead citrate-uranyl acetate and examined and photographed under electron microscopy.

Results

Diameters of the villi and fetal vasculature were also estimated by direct measurements. Normotensive and pre- eclamptic placentas were compared statistically and are shown in Table 1. Chorionic villi of 15 randomly selected villus diameter, vessel diameter, and the appreciation of statistics were performed by measuring the distance from the intervillous. Chorionic villi and vessel diameters were increased significantly in the pre-eclamptic group. Histopathologically, villous fibrin deposition outside the area showed an increase in syncytial nodes (Figure 1). The chorionic villi remained unaltered in the control group (Figure 2). Hypertrophy of cytotrophoblast cells, villous congestion, and dilation of blood vessels was also observed. Pre-eclamptic group in another section, stromal cell infiltration, and intervillous area commonly seen in the increase in the erythrocytes. Immunostaining with CD34 in the pre-eclamptic group compared to the control group showed a significant increase in the vessel wall (Figure 3). Ultrastructural examination revealed degeneration of mitochondria in endothelial cells, dilation of endoplasmic reticulum, and an increase of nuclei in the structure of chromatin (Figure 4). In the control group, the ultrastructural appearance of the normal chorionic villi can be seen in Figure 5. CD34 immunoreactivity in tissues from normal and pre-eclamptic pregnancies. Dark brown color of endothelial cells indicated positive staining for CD34 in villous core vessel endothelium (Figure 3). Pre-eclampsia was significantly increased as a result of the activity of CD34 in endothelial cells.

Fig. 3a



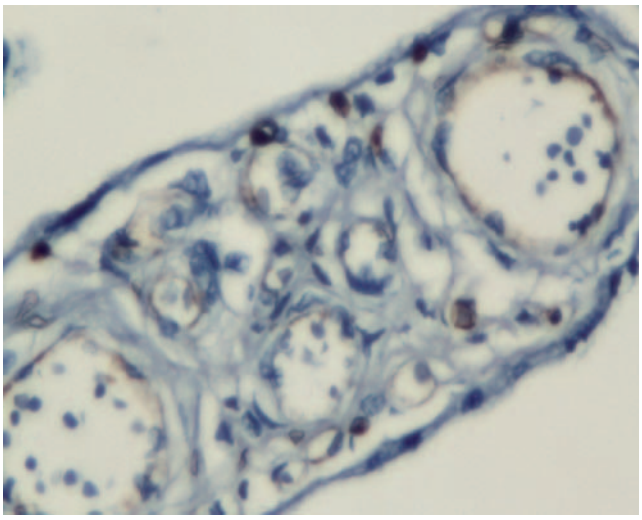
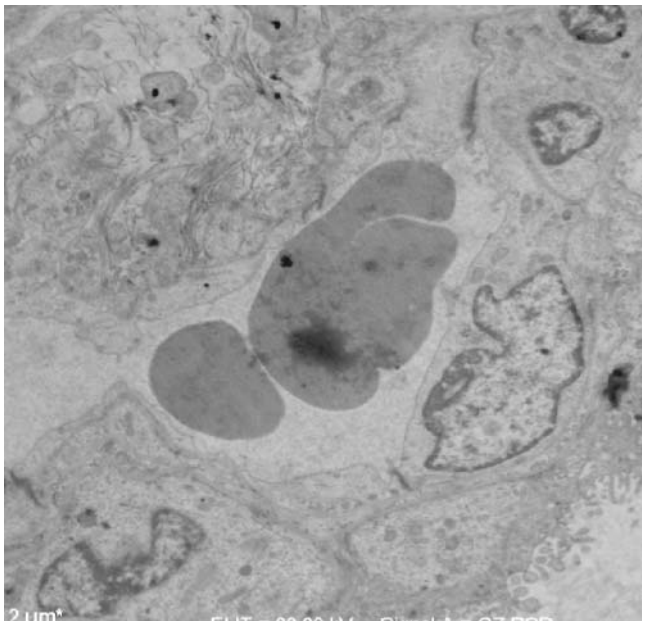


Fig. 3b

Figure 3. — Positive CD34 staining in villi core vessel endothelium. CD34 immunoreactivity in pre-eclamptic group; (b) CD34 immunoreactivity in control group.

Fig. 4



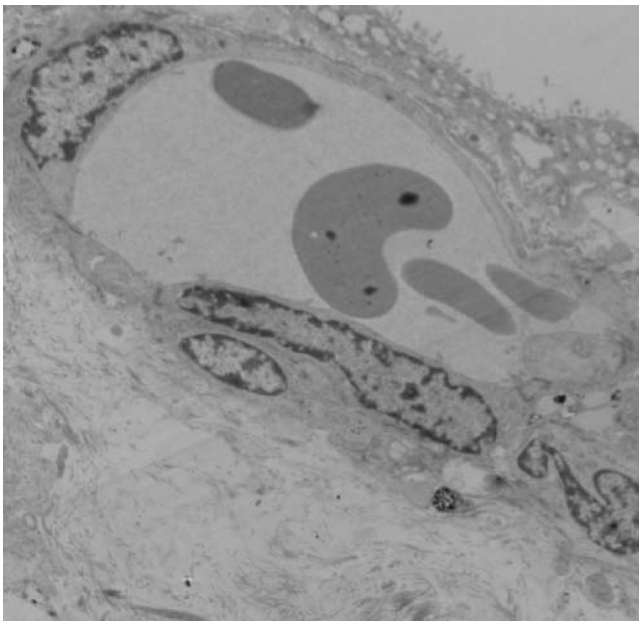


Fig. 5

Figure 4. — Chorionic villus section (pre-eclamptic group) showing vascular endothelial cell degeneration in mitochondria (arrow) an increase of chromatin and of extracellular matrix (uranyl-acetate X 8600).
Figure 5. — Chorionic villus appearance of the control group (uranyl-acetate X 6800).

Discussion

In pre-eclampsia there may be vascular endothelial cell damage and dysfunction. Placental pre-eclampsia is characterized by an hypoxic placenta subjected to oxidative stress, while maternal pre-eclampsia arises from the interaction between a normal placenta and a maternal system susceptible or suffering from microvascular diseases, as well as long-term hypertension and/or diabetes [7]. Cytokines, growth factors, and adhesion molecules have been proposed as important mediators for successful placentation, as well as endothelial dysfunction [8]. Vasoconstriction in the development of pre-eclampsia, insufficient spiral artery invasion, trophoblastic vasoactive substances of endothelial origin, and other factors playing a role in the expander have been reported [9-12]. In case of maternal hypertension in pre eclamptic women, fetal vessels react with a general vascular constriction [13], which increases the peripheral resistances.

CD34 marks the endothelial cytoplasm and consequently, the surface of the fetal blood exchange with the maternal medium. Endothelial cell damage causing leakage in the cell membrane protein also commonly leads to a loss of integrity. Indicators of this situation in women with pre-eclampsia are proteinuria and the

development of peripheral and pulmonary edema [14]. Effects of nutrition and metabolic changes in fetal placental vascular bed could be due to the terminal chorionic villi vascularization which plays a very important role in pregnancy. As a result of pre-eclampsia, histopathological changes in villus vessels, as well as degeneration of vascular endothelial cells, and mitochondrial damage were observed. The etiology of pre-eclampsia could be due to decrease of platelets and endothelial cell damage. In the present study, the expression of CD34 in endothelial cells increased in the pre-eclamptic group and influenced the development of blood vessels. The vessel wall, as a result of this change, would be helpful in understanding the etiology of pre-eclampsia. Ultrastructural stage, degeneration of mitochondria to the cytoplasm, and organelles at the level of chromatin explain the increase in endothelial damage. As a result, dysfunction and vascular endothelial cells, in response to the development of intravascular coagulation, are thought to be effective in pre-eclamptic pathology.

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