

## Structural Changes of Placenta in Preeclamptic Patients: Light and Electron Microscopic Study

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**Background:** Preeclampsia is a disorder that occurs only during pregnancy and the postpartum period and affects both the mother and the unborn baby. It is a rapidly progressive condition characterized by high blood pressure and the presence of protein in the urine. The study was designed to investigate the histological and ultrastructural changes in placentas from pregnancies complicated by preeclampsia.

**Methods:** Paraffin sections from placenta biopsies were prepared for light microscopic examination. For ultrastructural examination, biopsies were prepared and examined by digital scanning electron microscope.

**Results:** Light microscopic examinations showed that the nuclei of syncytiotrophoblast layer were aggregated into clusters in numerous sprouts and in long anastomosing strands. Villous connective tissue core was progressively condensed while the fetal placental capillaries regressed up to complete disappearance. Endothelial degenerative and atheromatous changes were seen in placental stem vessels, also basal decidual arterioles showed endothelial degeneration, progressive fibrosis, and obliteration. By SEM, villous tissues from the preeclamptic cases demonstrated elongated villi with wrinkled surfaces and covered with fibrin-like plaques. Capillary loops in preeclamptic cases were sparse in number and significantly longer compared to the control cases. They exhibited fewer branches and majority of the loops were uncoiled.

**Conclusions:** In placentas complicated by preeclampsia, ischemic damage of placental tissue with maldeveloped terminal villi occurs. These findings are consistent with an increase in fetoplacental vascular impedance where absent end-diastolic flow velocity was demonstrated in umbilical artery before delivery. These findings account for impaired gas and nutrient transfer in this disorder.

**Key Words:** Histology, placenta, preeclamptic patients

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### Preeklamptik Hasta Plasentalarında Yapısal Değişiklikler: Bir Işık ve Elektron Mikroskopi Çalışması

**Giriş:** Preeklampsia, sadece hamilelik sırasında ve postpartum döneminde görülen ve hem anne hem de cenin üzerinde etkisi olan bir bozukluktur. Çok hızlı gelişen bu bozukluk, yüksek tansiyon ve idrarda protein bulgusuyla karakterize edilir. Bu çalışma preeklampsili hamileliklerdeki plasentalarda oluşan histolojik ve yapısal değişiklikleri araştırmak üzere yapılmıştır.

**Yöntemler:** Placenta biyopsilerinden elde edilen paraffin kesintileri ışık mikroskopunda incelenmek üzere hazırlandı. Biyopsiler yapısal inceleme amacıyla dijital tarama electron mikroskopunda değerlendirildi.

**Bulgular:** Işık mikroskobu incelemesinde sinsiyotrofoblast katmanı çekirdeğinin pekçok filizlerde anastomoz yapan uzun katmanlar oluşturduğu gözlemlendi. Cenin plasentasındaki kılcal damarlar görünmeyecek kadar geriye çekilirken, villus bağ doku çekirdeği ileri derecede yoğunlaşmıştı. Plasental kord damarlarında endotelial dejeneratif ve ateromatöz değişiklikler ve aynı zamanda bazal desidual arteriyollerde endotelial dejenerasyon, progresif fibrosis ve obliterasyon gözlemlendi. SEM kullanılarak, preeklampsia vakalarındaki villus dokularında daha uzun villiler ve fibrin plakaları ile kaplı ve kırışık trofoblast yüzeyi olduğu saptandı. Preeklampsia vakalarında kılcal damar düğümlemesi çok sık olmamakla birlikte kontrol grubuna göre önemli derecede daha uzun olduğu gözlemlendi.

**Sonuç:** Preeklampsili hamileliklerdeki plasentalarda, placenta dokularında iskemiden kaynaklanan hasar sonucu gelişim anomalisi olan terminal viluslar meydana gelir. Bu bulgular, doğumdan önce göbek bağı arterinde end-diastolik akış hızı olmamasına rağmen fetoplacental damar içbasıncındaki yükselmeyi de açıklamaktadır. Bu bulgular bu hastalıktan kaynaklanan zayıf gaz ve besin transferine de açıklık getirmektedir.

**Anahtar Sözcükler:** Histoloji, placenta, preeklampsia hastaları

### Introduction

Normal fetal growth and survival depend on the proper development and function of the placenta, which serves to maintain a maternal-fetal interference for the exchange of blood gases, nutrients, and waste (1).

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Preeclampsia is a pathological condition due to the failure of proper placental development. It is a common gestational morbidity afflicting 7%–10% of all pregnancies and is a major cause of maternal and fetal morbidity and mortality; however, its etiology remains unclear (2,3).

The villous placenta undergoes constant renewal like most epithelia, and the syncytiotrophoblast is maintained by fusion with underlying cytotrophoblasts (4). In disorders like preeclampsia, cytotrophoblast invasion is limited to the superficial decidua, and few arterioles are breached due to abnormalities in adhesion molecule switching by invasive cytotrophoblasts, suggesting that this subpopulation of trophoblast cells fails to differentiate properly (5).

The study was designed to investigate the histological and ultrastructural changes in placentas from pregnancies complicated by preeclampsia.

## Materials and Methods

Twenty pregnancies (study group) complicated by severe intrauterine growth retardation (IUGR) were identified antenatally according to the following criteria: Estimated fetal weight <10<sup>th</sup> percentile and reduced amniotic fluid volume (defined by maximum vertical depth of cord-free amniotic fluid <3 cm). All the study group pregnancies were complicated by preeclampsia (defined by a blood pressure >140/100 mmHg on 2 or more occasions with a rise in diastolic blood pressure > 25 mmHg) occurring after 20 weeks of gestation together with proteinuria (0.3 g/24 h or  $\geq + 3$  on dipstick testing) (6). All members of the study group were delivered by planned cesarean section.

All neonates showed signs of IUGR. The control group (n = 20) included pregnancies with normal growth fetuses (with the same mean gestational age) as the study group, singleton pregnancies resulting in live born structurally normal neonates. All participants were from among the attendants of the obstetric department of AL-Gomhuria hospital, Cairo, Egypt (AGCE) and were recruited into the study after obtaining their informed consent. Tissue collection was approved by The Human Subject Research Committee of AGCE.

Pulsed Doppler recordings from the umbilical artery were performed with a Toshiba machine (Model 128 × B/10 ultrasound color Doppler: Toshiba Co. Ltd Tokyo,

Japan). After delivery, the placentas were weighed and examined from the maternal aspect to avoid sampling from areas of obvious infarction. Full-thickness samples were taken from the central part and preparation were performed as previously described (7). Samples were processed routinely for paraffin embedding, cut at 5-7  $\mu\text{m}$ , stained with hematoxylin and eosin (8). Scanning electron microscopic (SEM) investigations were done using digital SEM (Jeol 1200 EXII). Pieces of specimens were fixed in 2.5% glutaraldehyde at 4 °C for 2 h, rinsed with cacodylate buffer solution and post fixed in 2% osmium tetroxide. The specimens were dehydrated in graded concentrations of ethanol. A part of each specimen was mounted on a stub and spotted with gold (3 nm) for SEM investigation and other parts of these specimens were embedded in epoxy resin where semi-thin sections were prepared and stained with toluidine blue.

## Statistical analysis

Statistical analyses were performed using an unpaired Student's t-test. The data were analyzed using Excel 2000 (Microsoft, USA) and Sigma Plot 2001 (SPSS, USA).

## Results

The demographic and pregnancy characteristics of each group are presented in Table. Preeclamptic women and controls were similar in age and gestational age at delivery. However, the mean arterial pressure, birth weight, and placental weight of the preeclamptic women were significantly different than those of the control subjects ( $P < 0.01$ ).

In control placentas, light microscopic villous structures appeared with central connective tissues core covered by trophoblastic cell layers; each villous was rich with fetal capillaries. Also, villi were separated from each other by intervillous spaces filled with maternal blood (Figures 1 and 2).

Specific histological changes were seen in the preeclamptic placentas. Distribution of the nuclei in the syncytiotrophoblasts defined some changes with a tendency toward formation of clusters, (Figure 3) especially where the syncytial layer develops sprouts protruding into the intervillous spaces (Figure 3). Long slender syncytial strands bridge the intervillous spaces

connecting one villus to another and give the villous tree a pseudolabyrinthine appearance (Figures 3 and 4). Sectioned syncytial strands or sprouts are identifiable by the absence of any villous core (Figure 4).

The connective tissue villous stroma exhibit a great cellularity and fibrillar content leading to a prominent collagen staining affinity of the entire villous core (Figure 5). Fetal capillaries (Figure 5) have usually disappeared in most villi; however, fetal capillary remained recognizable occasionally (Figure 5).

Red blood corpuscles were identifiable in the lumina of a small number of the still preserved capillaries (Figure 5). The connective tissue core proliferated in the terminal villi replacing completely the fetal blood sinusoids (Figure 6). Even in the absence of any capillary wall structure, rare villi exhibited some fetal nucleated red blood cells seeming to lie

directly in the connective tissue stroma (Figure 7). Branches of the main umbilical vessels showed all stages of endothelial wall degeneration and atheromatous formation (Figures 8 and 9). Basal decidual arterioles showed endothelial degeneration with progressive fibrosis and obliteration (Figure 10).

Scanning electron microscopic appearances of terminal convolutes together with the corresponding loops from control placenta are shown in Figure 11. The corresponding pictures of the peripheral villous tree are shown in Figure 12. These terminal villous structures were characterized by a dense population of multiple small buds like villi containing projections, similar in size and shape, corresponding to sinusoidal dilatations at the tips of terminal capillary loops. Typical findings from preeclamptic cases are shown in Figure 13. Terminal

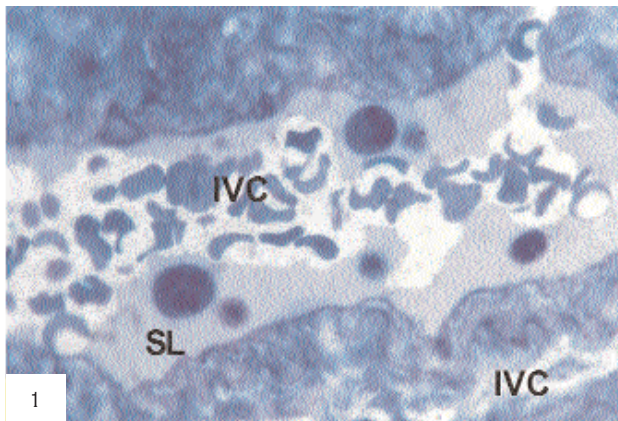


Figure 1. Villi of control placentas showing connective tissue core, syncytial cell layer (SL) covered by microvilli and intervillous space (IVS). (Toluidine blue,  $\times 250$ ).

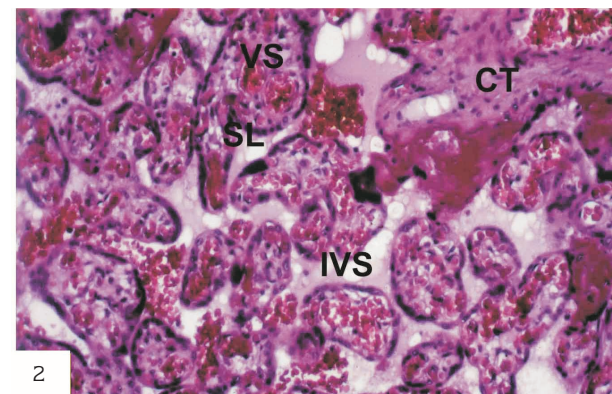


Figure 2. Villi of control placentas showing clusters of syncytial layer (SL), villous connective tissue core (CT), villous sinusoids (VS), and intervillous spaces (IVS). (H&E,  $\times 100$ ).

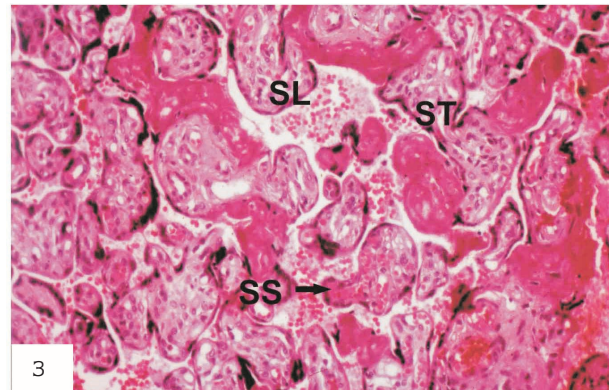


Figure 3. Villi of preeclamptic placentas showing clusters of syncytial layer (SL), syncytial sprouts (SS), and syncytial strands (ST) bridge the intervillous spaces. Sections are identifiable by the absence of any villous core. (H&E,  $\times 100$ ).

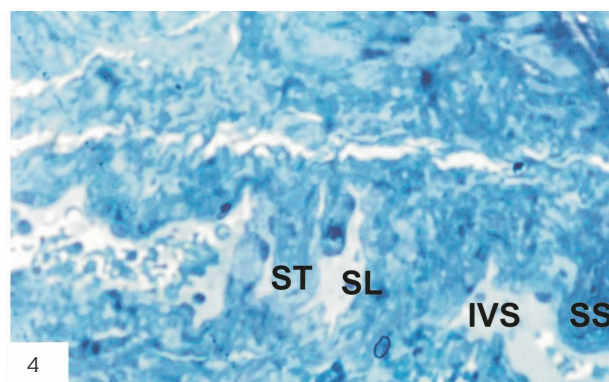


Figure 4. Villi of preeclamptic placentas showing clusters of syncytial layer (SL), syncytial sprouts (SS), and syncytial strands (ST). (Toluidine blue,  $\times 250$ ).



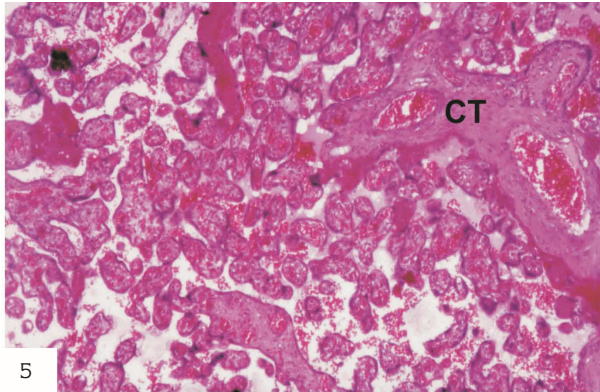


Figure 5. Villi of preeclamptic placentas showing condensed villous connective tissue core, regression of villous capillaries up to complete disappearance. (H&E,  $\times 100$ ).

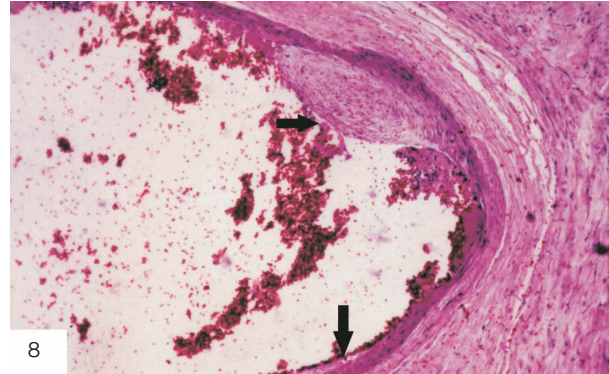


Figure 8. Villi of preeclamptic placentas showing umbilical vessels with endothelial degeneration and atheromatous formation. (H&E,  $\times 100$ ).

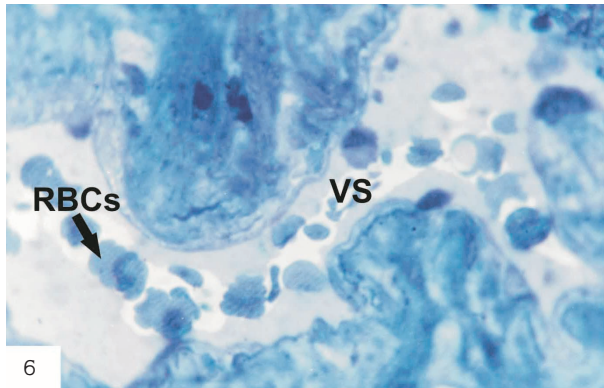


Figure 6. Villi of preeclamptic placentas showing red blood corpuscles in the lumen of the still preserved capillaries. (Toluidine blue,  $\times 100$ ).

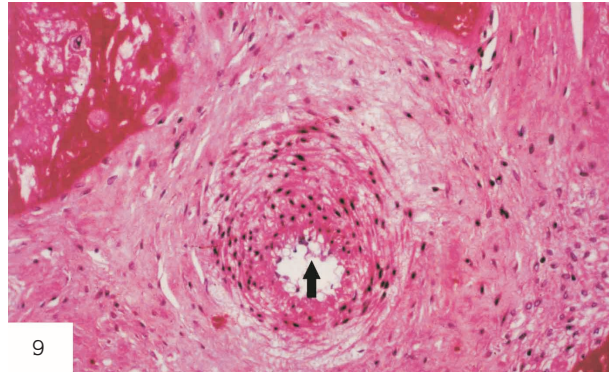


Figure 9. Photomicrograph of villi of preeclamptic placentas showing umbilical vessels with endothelial degeneration and gradual obliteration of their lumen. (H&E,  $\times 100$ ).

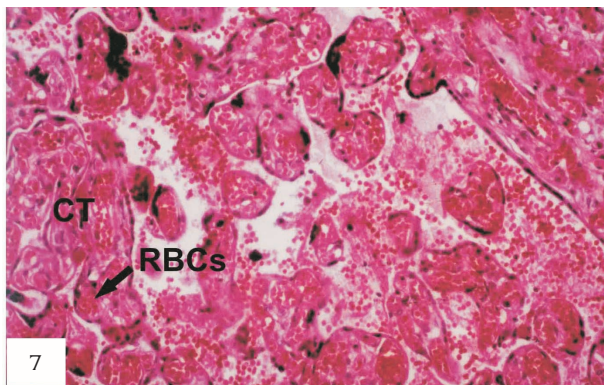


Figure 7. Villi of preeclamptic placentas. Notice the absence of capillary wall, RBCs directly in the connective tissue stroma (CT). (H&E,  $\times 100$ ).

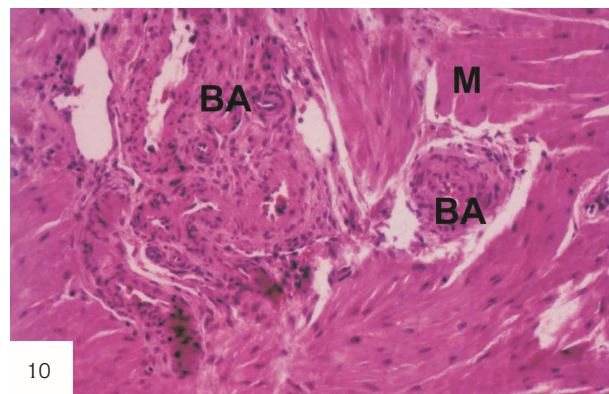


Figure 10. Photomicrograph of basal decidual arterioles (BA) showing endothelial degeneration, fibrosis, and obliteration. Myometrium appeared surrounding basal arterioles (M). (H&E,  $\times 100$ ).



Table. Demographic and pregnancy characters.

Variable	Control ± SD	PET ± SD	P-value
Maternal age (year)	30.4 ± 4.9	36.9 ± 5.6	NS
Gestational age (weeks)	31.3 ± 2.55	31.4 ± 2.48	NS
Maternal blood pressure (mmHg)			
Systolic*	118.1 ± 6.4	147.8 ± 7.3	< 0.01
Diastolic*	70.2 ± 9.5	103.5 ± 3.6	< 0.01
Birth weight (g)*	1808.4 ± 534	1204.3 ± 353.6	< 0.01
Placental weight (g)*	520.25 ± 114.2	261.0 ± 90.3	< 0.01
Umbilical artery Doppler results	RI, 0.69 ± 0.03	AEDFV	
Indication for delivery	9 CI, 9 PTL, 2 elective delivery	10 IUGR, 10 IUGR+PE	

Data are presented as mean ± SD \* Student's t-test was performed and a value of P < 0.01 was considered statistically significant. NS, not significant; AEDFV, absent end diastolic flow volume; RI, resistance index ; PTL, unexplained preterm labor; CI, cervical incompetence; PET, preeclampsia.

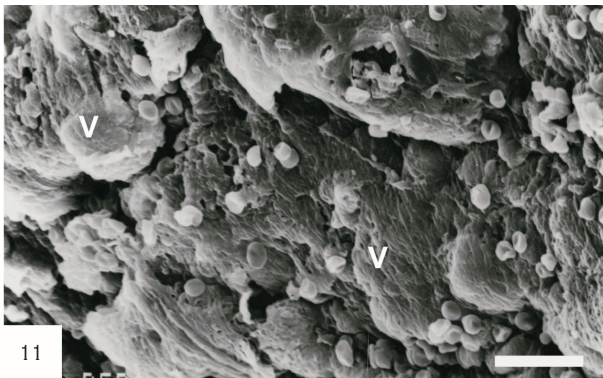


Figure 11. Scanning electron micrograph of the villi (V) of control placentas showing terminal convolutes with the corresponding loops. Scale bar: 10 µm.

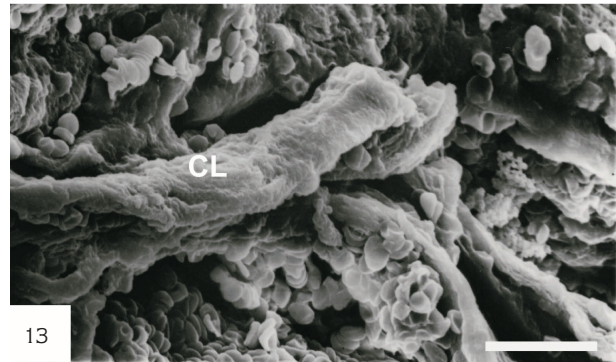


Figure 13. Scanning electron micrograph of the terminal villous structure of the preeclamptic placentas showing sparse capillary loops (CL), which are longer than control ones with no evidence of coiling. Scale bar: 10 µm.

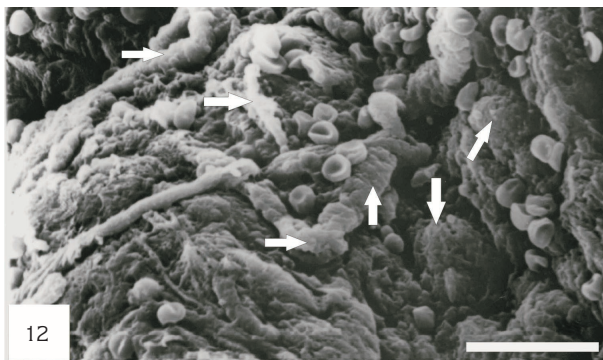


Figure 12. Scanning electron micrograph of the terminal villous structure (arrow) of control placentas showing bud-like villi containing projections similar in size and shape (sinusoidal dilatation). Scale bar: 10 µm.

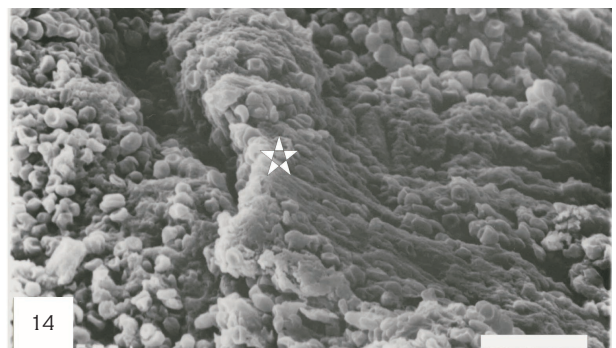


Figure 14. Scanning electron micrograph of the villi of preeclamptic placentas showing plaques of fibrin like material on syncytial surface of villous tissues with wrinkled appearance (star). Scale bar: 10 µm.

capillary loops were sparse in number and were longer compared to the control group. The elongated loops in the preeclamptic cases had significantly fewer branches and the majority of the capillary loops showed no evidence of coiling (Figure 13). Terminal villi were much fewer in number than the controls, and they had the appearance of elongated drainpipes (Figure 14).

Large plaques of fibrin like material often extending between several villi were present on the syncytial surface of villous tissues in preeclamptic cases. The surface covering the villi demonstrates a striking wrinkled appearance, forming folds, as if the trophoblast was piled up (Figure 14).

## Discussion

Villous growth during the first and early second trimesters is directed towards the formation of stem villi, after which the immature intermediate villi differentiate into their mature counterparts, directing growth toward the formation of nonmuscularized mature intermediate and terminal villi (9,10). Longitudinal growth of the capillaries within the mature intermediate villi exceeds that of the villi themselves so the capillaries coil and form loops that bulge from the villous surface, forming grape-like outgrowths known as terminal villi. Their capillary convolutes from a rich network of loops with many intercalated branches and random focal dilatation known as sinusoids (9,10). The development of this low impedance capillary network parallels the proportional rise in fetal cardiac output entering umbilical arteries to about 40% at term (11); this arrangement favors maximum nutrient and gas exchange as the fetus grows.

Our detailed studies of terminal villous ultrastructure in preeclamptic cases indicated increased occlusive process taking place within abnormally developed terminal villous capillaries, although deterioration in fetal cardiac function with progressive hypoxia and acidosis also contributed (6). Our observations of maternal thrombosis surrounding peripheral villi will also contribute to deteriorating placental gas exchange in preterm preeclampsia. The wrinkled appearance of the trophoblast in our preeclamptic cases is clearly abnormal; we have suggested that this may be due to reduced trophoblast turnover. Many of the metabolic functions of villous syncytiotrophoblast, such as the transport of amino acids or production of nitric acid, may be impaired

consequently because syncytiotrophoblast regeneration is dependent on new cytotrophoblast fusing into the syncytium (6). In the case of nitric oxide impaired expression within villous syncytiotrophoblast may facilitate the formation of maternal fibrin plaques on the surface of these abnormal villi.

In the present study, the affected placental villi manifested the condensed villous connective tissue core while fetal placental capillaries regressed up to disappearance. These findings account for impaired gas and nutrient transfer. Syncytial sprouts and long anastomosing strands in spite of regression of villous capillaries were present, increasing the surface area for fetomaternal exchanging. Our finding was in agreement with previous studies (12,13). Earlier studies suggested that these changes were caused by hypoxia (14).

Degenerative changes in the form of trophoblastic apoptosis were found in placentas from pregnancies complicated by fetal growth restriction resulting from hypoxia (15).

Our results showed that stem vessels appeared with atheromatous changes and basal decidual arterioles were progressively obliterated. The relative frequency of hypoxic changes and atherosclerosis in placentas in preeclamptic cases have been published before (16,17). The latter was histopathologically characterized by fibrinoid necrosis and perivascular mononuclear infiltration by lipophages. The etiology and pathogenesis of atherosclerosis are still unclear, but its similarity to vascular lesions in allograft rejection reaction, according to some authors, suggested the involvement of the immune system (18,19). The fact that it appears in vessels without physiological changes of pregnancy explains the more frequent formation of luminal obstruction or thrombosis and subsequent hypoxic changes of placental tissue. Alternations in its levels are associated with preeclamptic placental changes (20, 21).

The pathological basis of absent end-diastolic flow velocity was a constant finding in all pregnancies complicated by IUGR, which is widely assumed to be due to an obliterative process involving small stem villous arterioles because light and scanning microscopic studies demonstrated reduced density of vessels profile and arteriolar obstruction or thrombosis. These findings are consistent with the other findings (6,7).

In conclusion, this study showed that in placentas complicated by preeclampsia, ischemic damage of

placental tissue occurs with maldeveloped terminal villi. These findings are consistent with an increase in fetoplacental vascular impedance where absent end-diastolic flow velocity demonstrated in umbilical artery before delivery. These findings account for impaired gas and nutrient transfer in this disorder.

## References

1. Many L, Della Y, Terry RM, Oliver S, Kathleen MC. Reduced maternal expression of adrenomedullin disrupts fertility, placentation, and fetal growth in mice. *The journal of clinical investigation* 2006; 116: 2653-2662.
2. Granger P, Alexander BT, Bennett WA, Khalil RA. Pathophysiology of pregnancy-induced hypertension. *Am J Hypertens* 2001; 14: 178-185.
3. Luo ZC, An N, Xu HR, Larante A, Audibert F, Fraser WD. The effects and mechanisms of primiparity on the risk of preeclampsia: a systematic review. *Paediatric and Perinatal Epidemiology* 2007; 21: 36-45.
4. Kaufmann P, Stegner HE. Functional differentiation of the human placental syncytiotrophoblast. *Z Zellforsch Mikrosk Anat* 1972; 135: 361-382.
5. Kadyrov M, Kingdom JC, Huppertz B. Divergent trophoblast invasion and apoptosis in placental bed spiral arteries from pregnancies complicated by maternal anemia and early-onset preeclampsia/intrauterine growth restriction. *Am J Obstet Gynecol* 2006; 194: 557-563.
6. Macara L, Kingdom JC, Kaufmann P, Kohnen G, Hair J, More IA, Lyall F et al. Structural analysis of placental terminal villi from growth-restricted pregnancies with abnormal umbilical artery Doppler waveforms. *Placenta* 1996; 17: 37-48.
7. Krebs C, Vet M, Macura LM, Leiser R, Bowman AW, Gree IA et al. Intrauterine growth restriction with absent and diastolic flow velocity in the umbilical artery is associated with maldevelopment of the placental terminal villous tree. *Am J. of obstet. Gynecol* 1996; 175: 1534-1542.
8. Adam H, Caihak G. *Grosses zo-ologisches parktikum tell. Arbeitsmethoden der makroskopischen und mikroskopischen anatomic Mit 283 Ab-bildungen* Gustav. Stutt-gart: Fischer Verlag; 1964.
9. Kaufmann P, Bruns U, Leiser R, Luckhart M, Winterhager E. The fetal vascularization of term human placental villi, II: intermediate and terminal villi. *Anat Embryol* 1985; 173: 203-214.
10. Kaufmann P, Burton G. Anatomy and genesis of the placenta. In: Knobil E., Neill JD, editors. *The physiology of reproduction*. New York: Raven Press; 1994; 441-483.
11. Eik-Nes S, Brubakk AO, Ulstein M. Measurement of human fetal blood flow. *BMJ* 1980; 1: 283-284.
12. Allaire AD, Ballenger KA, Wells S R, McMahon MJ, Lessey BA. Placental apoptosis in preeclampsia. *Obstet Gynecol* 2000; 96: 271-276.
13. Ishihara N, Matsuo H, Murakoshi H, Laoag-Fernandez JB, Samoto T, Maruo T. Increased apoptosis in the syncytiotrophoblast in human term placentas complicated by either preeclampsia or intrauterine growth retardation. *Am J Obstet Gynecol* 2002; 186: 158-166.
14. Levy R, Smith SD, Chandler K, Sadovsky Y, Nelson DM. Apoptosis in human cultured trophoblasts is enhanced by hypoxia and diminished by epidermal growth factor. *Am J Physiol Cell Physiol* 2000; 278: 982-988.
15. Hu R, Zhou S, Li X. Altered Bcl-2 and Bax expression is associated with cultured first trimester human cytotrophoblasts apoptosis induced by hypoxia. *Life Sci* 2006; 79: 351-355.
16. Benirschke K, Kaufmann P. *Pathology of the human placenta*. 2nd ed. New York: Springer Verlag; 1990.
17. Moldenhauer JS, Stanek J, Warshak C, Khoury J, Sibai B. The frequency and severity of placental findings in women with preeclampsia are gestational age dependent. *Am J Obstet Gynecol* 2003; 189: 1173-1177.
18. Stallmach T, Hebisch G, Orban P, Lu X. Aberrant positioning of trophoblast and lymphocytes in the feto-maternal interface with pre-eclampsia. *Virchows Arch* 1999; 434: 207-211.
19. King A, Burrows TD, Hiby SE, Bowen JM, Joseph S, Verma S et al. Surface expression of HLA-C antigen by human extravillous trophoblast. *Placenta* 2000; 21: 376-87.
20. Dey S K, Lim H, Das SK, Reese J, Paria BC, Daikoku T et al. Molecular cues to implantation. *Endocr Rev* 2004; 25: 341-373.
21. Nikitenko LL, Blucher N, Fox SB, Bicknell R, Smith DM, Rees MC. Adrenomedullin and CGRP interact with endogenous calcitonin-receptor-like receptor in endothelial cells and induce its desensitisation by different mechanisms. *J Cell Sci* 2006; 119: 910-922.

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