Evaluation of Trophoblast Invasion in Placental Bed Biopsies of Women with Normal and Severe Preeclamptic Pregnancies

NORMAL GEBELİK VE ŞİDDETLİ PREEKLAMPTİK GEBELİKLERDE PLASENTAL YATAK BİYOPSİLERİNDE TROFOBLAST İNVAZYONUNUN DEĞERLENDİRİLMESİ

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Summary

This study was designed to evaluate the trophoblast invasion and vascular changes in placental bed spiral arteries in normal and severe preeclamptic pregnancies.

Placental bed biopsies from 16 women with normal pregnancy and 17 women with severe preeclamptic pregnancy were included in this study. Trophoblast invasion to decidual and myometrial spiral arteries as well as vascular changes such as acute atherosis and medial hyperplasia were investigated histopathologically, histochemically and immunohistochemically.

In normal pregnancy group, trophoblast invasion in both decidual and myometrial segments was observed in 14 (87%) of the 16 women. However, in severe preeclamptic pregnancy group, 11 (64%) of the 17 women had decidual trophoblast invasion and only five (29%) of 17 had both decidual and myometrial trophoblast invasion. Acute atherosis was not observed. Medial hyperplasia was determined in four (28%) of 14 pregnancies.

The extent of trophoblast invasion in myometrial spiral arteries in severe preeclamptic women is significantly lower than that of women with normal pregnancies. Inadequate trophoblastic invasion seems to play one of the important roles in the pathogenesis of preeclampsia.

Key Words: Preeclampsia, Trophoblast, Invasion, Immunohistochemistry

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Özet

Bu çalışmada normal ve şiddetli preeklamptik gebelerde trofoblastik invazyon ve spiral arterlerde oluşan değişiklikler değerlendirildi.

Çalışmada 16 normal gebeden ve 17 şiddetli preeklamptik gebeden plasental yatak biopsisi alındı. Desidual vc myometrial spiral arterlerdeki trofoblastik invazyon, akut aterozis ve medial hiperplazi gibi vasküler değişiklikler histopatolojik, histokimyasal ve immünohistokimyasal olarak incelendi.

Normal gebe grubunda desidual ve myometrial trofoblastik invazyon 16 olgunun 14'ünde (%87) gözlendi. Şiddetli preeklamptik gebelik grubunda 17 kadının 11 'inde (%64) desidual trofoblastik invazyon, 5'inde (%29) hem desidual hemde myometrial trofoblastik invasyon bulundu. Spiral arterlerde akut aterozis görülmedi. Medial hiperplazi 14 gebeliğin 4'ünde (%28) saptandı.

Şiddetli preeklamptik gebelerde myometrial spiral arterlerdeki trophoblast invazyonu normal gebeliklerlere göre belirgin derecede daha az olmaktadır. Yetersiz trofoblast invazyonu preeklampsi patogenezinde rol oynayan önemli faktörlerden biridir.

Anahtar Kelimeler: Preeklampsi, Trofoblast, İnvazyon, İmmünhistokimya

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Preeclampsia occurs in 7-10% of pregnancies and remains an important cause of maternal-fetal morbidity and mortality (1). The placenta plays a central role in the pathogenesis of preeclampsia, as removal of this organ at delivery results in rapid resolution of the disease (2-5). Shallow endovascular cytotrophoblast invasion in the spiral arteries and endothelial cell dysfunction are two key features in the pathogenesis of preeclampsia, yet the etiology of preeclampsia is still unknown (1).

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During the early weeks of gestation, cytotrophoblast cells stream out of the tips of the anchoring villi and penetrate the trophoblast shell and the overlying syncytiotrophoblast to form cytotrophoblast columns that develop into the cytotrophoblast shell. Trophoblast cells continue to migrate into the decidua and eventually colonize the placental bed's myometrium. When the cytotrophoblast shell contacts the spiral arteries' openings, trophoblast cells stream into their lumina, where they form intraluminal plugs. Cytotrophoblast plugs may act as valves regulating blood flow in the intervillous space and protect the embryo from forceful maternal blood flow. In normal pregnancies, trophoblast cells replace the endothelium of spiral arteries of both decidual and myometrial segments and then invade the media, with resulting destruction of the medial elastic, muscular, and neural tissue. Trophoblast cells become incorporated into the vessel wall, and in the final stages, the endothelial lining is reconstituted. In this circumstance, trophoblast cells are called endovascular trophoblast. These physiologic changes create a low-resistance arteriolar system and an absence of maternal vasomotor control, which allows the dramatic increase in blood supply to the growing fetus (1).

In preeclampsia, it is believed that trophoblasts do not invade the myometrial segments and physiological change is restricted to decidual segments (6-9, 10-12) In these pregnancies acute atherosis that means distinctive lesion of uterine spiral arteries accompanied by foam cell infiltration is likely to develop in the myometrial spiral arteries (6-8, 13).

This study was undertaken in an attempt to clarify the extent of extravillous trophoblast invasion in the decidual and myometrial segment of the spiral arteries in women with normal and severe preeclamptic pregnancies and also to evaluate vascular morphological changes such as acute atherosis and medial hyperplasia seen in severe preeclamptic pregnancies.

Material and Methods

The study groups included 17 women with severe preeclampsia according to the definition of ACOG (American College of Obstetricians and Gynecologists) (14) and 16 women with normal term pregnancy. The American College of Obstetricians and Gynecologists defines severe preeclampsia as the presence of one or more of the following clinical manifestations: 1) a systolic blood pressure 160 mm Hg or a diastolic blood pressure 110 mm Hg on two occasions at least 6 hours apart, 2) proteinuria of 5 g in a 24-hour period (or 3 to 4 based on semiquantitative analysis), 3) oliguria, a 24-hour urinary output <400 to 500 ml, 4) cerebral or visual disturbances, 5) pulmonary edema or cyanosis, 6) epigastric or right upper quadrant pain, 7) impaired liver function of unclear etiology, and/or 8) thrombocytopenia. All the women with severe preeclampsia underwent cesarean section with indications including vaginal bleeding, non-reassuring fetal status, eclampsia, uncontrolled severe hypertension, pulmoner edema, compromised renal function, persistent severe headache or visual changes, platelet count <100.000/mm^3, or aspartate aminotransferase or alanine aminotransferase value more than twice the upper limit of normal, with epigastric pain or right upper-quadrant tenderness. The study group with normal pregnancy also underwent cesarean section with other obstetric indications such as cephalopelvic disproportion, acute fetal distress and previous cesarean section.

With informed consent of the patients, the placental bed biopsies were taken under direct vision with scalpel during cesarean section. We obtained two or three biopsies of at least 1 cm^3 and placed them in buffered formalin. After fixation, dehydration and embedding in paraffin wax serial sections were cut at 3 Lim and stained with haematoxylin and eosin (H&E) and Von Giesson. Biopsies were included the study if at least one spiral artery is present.

In examination, decidual and myometrial segments were investigated separately. Trophoblast invasion was reported depending on whether it was embedded in the vessel wall. If there were at least one spiral artery in both myometrial and decidual segments which showed endovascular trophoblast invasion, it was recorded as decidual spiral arterial invasion and myometrial spiral arterial invasion separately. Immunohistochemical study was performed to distinguish trophoblasts from decidual cells without difficulty. Avidin biotin peroxidase complex (ABC) method was applied (15) and low molecular weight cytokeratin (Dako, USA) was used as primary antibody. Also the extent of vascular changes were de-

scribed. Atherosis and medial hyperplasia were recorded. Atherosis was determined by presence of foam cells and fibrosis in the vessel wall. Muscular tissue was identified by Von Giesson staining.

The following parameters were recorded: age, gestational week, parity, presence or absence of endovascular trophoblast invasion in decidual segments, presence or absence of trophoblast invasion in myometrial segments, presence or absence of acute atherosis and medial hyperplasia of spiral arteries. Data were analyzed by $\%^2$ test and Fisher's exact test when appropriate and a p value < 0.05 was regarded as significant.

Results

Table 1 shows the demographic data of patients with severe preeclampsia and normal pregnancy. There were no differences in age and parity in the study groups ($p > 0.05$), but gestational week of women with severe preeclampsia is significantly lower than that of women with normal pregnancies ($p<0.05$). In normal pregnancy trophoblast invasion both in decidual and myometrial spiral arteries was seen in 14 (87%) of 16 biopsies (Figure 1). In severe preec-lampsia although trophoblast invasion in decidual spiral arteries was seen in 11 (64%) of 17 cases, only 5 (29%) of 17 cases revealed myometrial invasion (Tablo 2). Totally 3 biopsies, 2 of which belongs to normal pregnancy group and one belongs to severe preeclamptic group did not contain trophoblasts neither in the decidual nor in myometrial segments of spiral arteries (Figure 2). The percentage of trophoblast invasion in myometrial spiral arteries in severe preeclamptic women was significantly lo-wer than those of women with normal pregnancies ($p<0.05$). However, between two groups statistically significant difference was not found in decidual arter invasion by trophoblasts ($p>0.05$) (Figure 3).

Table 1. Characteristics of women with normal and severe preeclamptic pregnancy

	Normal	Preeclampsia	Significance
No.	16	17	
Age (y)			
Median	26.12	26.64	NS
Range	18-39	20-32	*i*
Gestation (wk)			
Median	39	34	$p<0.05$
Range	37-41	31-38	
Primiparous (No.)	7	9	NS

NS, not significant

Immunohistochemical staining intensity were different (from weakly to strongly positive) but cases with trophoblast invasion were cytokeratin positive. No obvious differences were seen between normal and severe preeclamptic pregnancies in trophoblast staining characteristics. An area of acute atherosis in the vessel wall was not described in our study. In 4 (28%) of 14 biopsies in women with severe preeclampsia, medial hyperplasia in the wall of spiral arteries was detennined.

Discussion

In 1958, some authors introduced a new technique for obtaining tissue from pregnant uterus at caesarean section, which they called the placental bed biopsy. They wanted to emphasize that the biopsy must include both basal decidua and underlying myometrium contained uteroplacental arteries (7). These vessels thought to be the target organ of placenta in abnormal pregnancy such as hypertension, intrauterine growth retardation and preeclampsia (6,7). The most important feature in taking this biopsy is that they have to be obtained from central zone of the placental bed. Therefore, if the specimens do not contain extravillous tro-

Table 2. Trophoblastic invasion in women with preeclampsia and normal pregnancy

Trophoblastic invasion	Normal pregnancy (n=16)	Preeclampsia (n=17)	Statistical significance
Desidua and myometrium			
Positive (%)	14	5	$P<0.01$
Negative (%)	1 '	12	
Only desidual			
Positive (%)	14	11	$p>0.05$
Negative (%)	2	6	

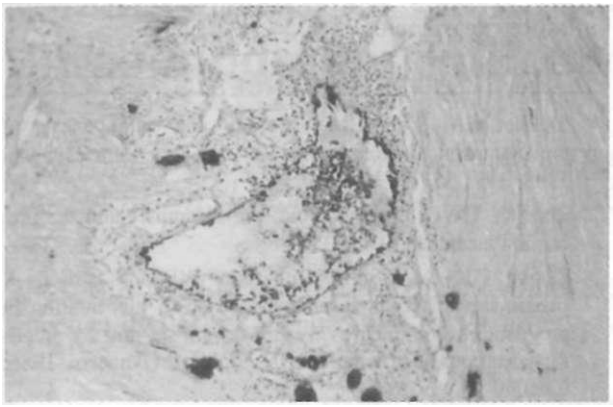

Figure 1. Trophoblasts, around and in the endotelial layer of the myometrial spiral artery (!HCx50).

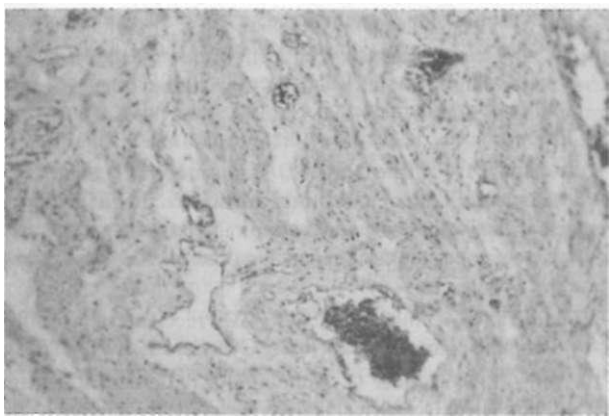

Figure 2. Normal myometrial spiral artery (IHCxlOO).

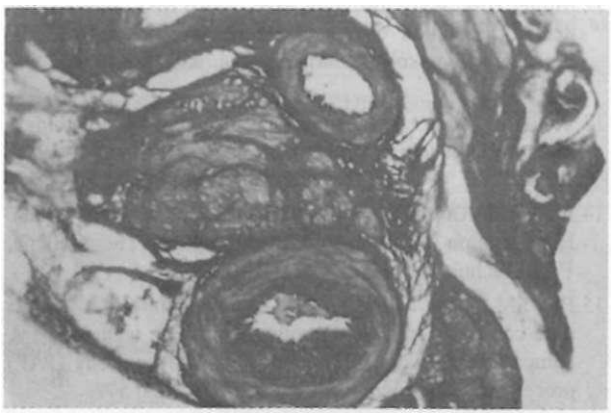

Figure 3. Spiral artery with medial hyperplasia (VGx50).

phoblast in the myometrium and in the decidua it must be thought that it is the marginal zone which is not representative for placental bed especially for spiral arteries (7,13). It is reported that in fact the placental bed is difficult to identify even at cesarean section and a substantial biopsy may not include a vessel (13). In this study in both group totally 3 biopsies did not contain any extravillous trophoblast neither in decidua nor in the myometrium. Therefore, it is thought that these biopsies may have been taken from the marginal zone of the placental bed.

Although the precise etiology of preeclampsia is not well known and complex, some authors demonstrated the role of trophoblasts in preeclampsia. They proposed that in preeclampsia there was not physiologic trophoblast invasion of the deep maternal spiral arteries (6,9,11,12). According to these authors inadequate trophoblastic invasion leads to vascular spasm and restricted blood flow to the placental fetal unit (6,7,9). In a study, 100 uteroplacental arteries were investigated and significant proportion of endovascular trophoblast migration is to be found escape from or resist involvement in the uteroplacental arteries in preeclampsia (7). However, some other authors do not support this theory. Because they found that in preeclampsia invasion into the myometrial segments can occur and also there could be non-pregnant structure in spiral arteries of these pregnancies. They propose that endovascular trophoblast invasion within a vessel wall may be variable for example, they may be restricted to one area or present in the whole segment (6,8).

Standard techniques may not be adequate to reveal trophoblasts. Thus to detect trophoblasts and differentiate them from decidual cells, IHC techniques may be required. Antibodies directed against keratin human plasental lactogen and human chorionic gonodotropin have been sensitive in detecting trophoblasts. Low molecular cytokeratin is the most common used antibodies directed against keratin (6,11,16).

In the present study, to identify trophoblasts easily, L M W cytokeratin was performed to all cases. The trophoblast invasion in myometrial spiral arteries in normal pregnancies was significantly higher than that of women with preeclampsia. In accordance with the reported studies (6-8), the cases in whom trophoblast invasion restricted to de-

cidual segments, demonstrated that endovascular trophoblast may have non-invasive phenomenon and physiological changes may be restricted to decidual segments in preeclampsia.

Acute atherosis, described as distinctive arterial lesion, was first described by Hertig in 1945 (7). Since that time there have been many studies of this arterial lesion mainly in relation to preeclampsia (6-8,10). It occurs only in those arteries that have not undergone physiological change mediated by endovascular trophoblast (6,8,13). It is believed that the development of this lesion in these pregnancies is because musculoelastic tissue remains intact (6,8,10). This precludes further vascular dilatation and increased placental perfusion which are essential for normal fetal growth (6,8). Hyperplasia of the muscular media in preeclampsia is the other common feature of spiral arteries (6,7). It is thought that in preeclampsia retaining their medial smooth muscle is responsible for the development of medial hyperplasia in myometrial spiral arteries. And medial hyperplasia was to be found the commonest feature of vascular change especially in the myometrial segments in preeclampsia. They act as a protective mechanism against high-pressure blood flow (6).

In this study, acute atherosis in the vessel wall was not observed in both groups. But medial hyperplasia was found in 4 of 17 cases in preeclampsia but none in normal pregnancies. In the cases with medial hyperplasia, there was no trophoblasts invasion. In our study the findings related to vascular changes were in agreement with reported studies except for atherotic change. We thought that medial hyperplasia might have been early sign of atherosis and if the pregnancy was not over in earlier period atherosis might have been developed in further weeks. In fact, it is so difficult to make such a conclusion with the vascular changes unless more spiral arteries are examined.

Although our study was undertaken in restricted number of cases we observed that there was a significant difference in endovascular trophoblast invasion between women with normal and severe preeclamptic pregnancies and this may be concluded this non-invasive phenomenon and defective physiological change may be a responsible factor for preeclampsia. However in order to support this idea more clinical and histopathological studies must be undertaken in more representative biopsies in more pregnancies.

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