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Pathological Changes in Placentas from Pregnancies with Preeclampsia and Eclampsia with Emphasis on Persistence of Endovascular Trophoblastic Plugs

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Aim To assess the frequency and types of histopathological changes in placentas from pregnancies complicated by preeclampsia/eclampsia.

Methods Placentas routinely sent for pathological examination (n = 1,689) were studied microscopically and compared to findings of 50 placentas from uncomplicated pregnancies.

Results Out of 1,689 placentas from singleton pregnancies, 279 (16.5%) were from pregnancies complicated by preeclampsia/eclampsia. Seventy five placentas (26.8%) were appropriate for gestational age; other findings included: infarcts of various stage and volume in 63 cases (22.6%), minimal hypoxic damage in 27 cases (9.7%), accelerated maturation in 42 cases (15.1%), chronic villitis in 18 cases (6.5%), mixed findings in 18 cases (6.5%), intervillous thrombosis in 15 cases (5.4%), subchorial thrombosis in 9 cases (3.2%), immaturity of the villi in 6 cases (2.1%), and findings suggestive of placental insufficiency in 6 cases (2.1%). Normal findings were significantly more frequent in the control group ($P < 0.001$), but no other significant differences between the groups were found. In 4 (1.4%) placentas from pregnancies complicated by preeclampsia/eclampsia (gestational age 32 to 36 weeks), remnants of endovascular trophoblastic plugs in the vessels of the basal decidua were found.

Conclusion No significant difference was found between the group of placentas from pregnancies complicated with preeclampsia/eclampsia and the control group with regard to ischemic changes of the placenta. Endovascular trophoblastic plugs in the basal plate vessels from the third trimester placentas may play an additional role in the development of ischemic lesions in the placentas from pregnancies complicated with preeclampsia/eclampsia, but may also simply represent indirect evidence of the abnormal expression of certain adhesion molecules in this disorder.

During the physiological changes that occur in the first and in the beginning of the second trimester of pregnancy, spiral arteries of the placental bed are converted into the uteroplacental arteries (1-3). The essence of this conversion consists of losing the muscular elements in the vessel walls, making them unable to respond to vasomotor influences. Cells that infiltrate the walls of spiral arteries and replace their normal elements are called migratory, non-villous, or intermediate trophoblastic cells (3,4). Beside infiltrating and replacing the anatomic structures of spiral arteries, intermediate trophoblastic cells also penetrate into the lumina of these vessels, forming endovascular plugs. These plugs are one of the reasons why early uteroplacental blood flow cannot be visualized, even with transvaginal ultrasound, during the first 12 weeks of gestation (5). In uncomplicated pregnancies, the endovascular trophoblast is bound to disappear by the end of the second tri-

mester of pregnancy, but the literature about this topic is scarce (6). Some studies, especially those dealing with the morphological analysis of placental bed, have recorded a partial or complete lack of physiological changes (ie replacement of just a part of vessel wall circumference, or findings of physiological changes only in decidual, but not in the inner myometrial portion of spiral arterioles) in uteroplacental vessels in pregnancies complicated by preeclampsia or eclampsia (7-9). In some vessels that have not undergone physiological changes, acute atherosis was noticed (7-10). When the whole placentas are examined, these changes are also noticed in the basal plate and especially in the amniochorial membranes (because physiological changes in the blood vessels are not developed there, due to the lesser degree of invasion of intermediate trophoblast) (9).

Here we describe another pathomorphological feature, persistent endovascular trophoblastic plugs. It can sometimes be observed in vessels of the placental bed of third trimester placentas, in pregnancies complicated by preeclampsia and eclampsia.

Material and Methods

During 1998-2002, 1,689 placentas from singleton pregnancies were consecutively sent for routine pathological examination. In 279 cases, the placentas were examined by a pathologist because of preeclampsia or eclampsia, defined by the obstetricians as systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mmHg (18.7 kPa or 12.0 kPa, respectively) measured at least twice and proteinuria of 300 mg/24 h or 300 mg/L with or without edema. The placentas were weighted immediately after delivery and examined by a pathologist (M. K. or Lj. H.), sectioned serially at 5-10 mm intervals, and 5-10 samples of the full placental thickness were taken for histopathological examination, as well as the sample of placental membranes. The membranes were stripped from the placental surface, and rolled so that the transverse cut for histologic sample was obtained. Two samples of the umbilical cord (from the placental and fetal ends) were also taken for histological examination. The samples were processed routinely, for paraffin embedding, cut at 5 μm, stained with hematoxylin-eosin and periodic acid-Schiff (PAS) stains, and examined by light microscopy (M. K. and Lj. H.). Histopathological findings were roughly divided into those adequate for gestational age, those with infarcts (of different age and extension), minimal hypoxic damage (characterized by circular cytotrophoblastic proliferation, basal membrane thickening, and excessive number of syncytial knots at the surface of most chorionic villi), accelerated maturation of placental villi, chronic villitis, mixed histological findings (normal villi, those showing accelerated maturation and edematous villi), intervillous thrombosis, subchorial thrombosis, immaturity of the villi, and findings suggestive of placental insufficiency (unexplained villous fibrosis combined with any of the above mentioned findings). Results of the pathological examination were compared with a control group of consecutively examined 50 placentas from uncomplicated singleton pregnancies with gestational age ranging from 29 to 40 weeks. In cases of premature delivery, all placentas showing signs of inflammation of the membranes were excluded from the study, which left only placentas from premature deliveries due to cervical insufficiency or otherwise unexplained cause. The maturity of the placental tissue was assessed according to gestational age.

For statistical analysis, χ^2-test was used. We used statistical software Analyse-it, version 1.71 (Analyse-it Software, Ltd, Leeds, UK) for Microsoft Excel for Windows.

Results

We analyzed 279 placentas from pregnancies complicated by preeclampsia or eclampsia. They constituted 16.5% of 1,689 consecutively routinely examined placentas during the period from 1998 to 2002. In 127 cases (45.5%), the diagnosis of hypertensive disorder of pregnancy was not the only one; 63 (22.6%) of the total number of placentas were also associated with the diagnosis of intrauterine growth retardation (IUGR) of the fetus. The mean gestational age was 36.4 ± 3.5 weeks, whereas in control group it was 38.2 ± 2.7 weeks ($\chi^2_1 = 0.503$, $P = 0.478$). Six placentas (2.2%) were from pregnancies ending before 28 gestational weeks; 120 placentas (43%) were from pregnancies ending between 28 and 37 gestational weeks, and 153 placentas (54.8%) were from term pregnancies. The mean placental weight was 483.4 ± 173.4 g, compared with 512.5 ± 83.1 g in the control group ($\chi^2 = 0.544$, $P = 0.393$). Figure 1 shows the histopathological

findings of placentas from pregnancies complicated by hypertensive disorders of pregnancy in comparison with the findings in the control group. In the group of normal placentas, the number of normal findings was more frequent than in the group of placentas from pregnancies complicated by preeclampsia/eclampsia ($P<0.001$). The difference between the number of other, even ischemic changes between the investigated and the control group was not significant. On gross examination, retroplacental hematoma was observed in 3/279 (1.07%) placentas. Acute atherosis of uteroplacental vessels was found in 8 cases (2.8%). In 3 cases, it was localized in placental membranes, and in 5 cases in the basal decidua. In 4/279 (1.4%) placentas, from gestation of 32 weeks and 4 days to 36 weeks and 2 days, trophoblastic plugs in the vessels of the basal decidua were found, either attached to the vessel wall (Fig. 2) or seemingly free in the lumen (Fig. 3). In all 4 cases, the pregnancy was complicated only by preeclampsia, and the overlying placental villi showed evidence of cytotrophoblastic proliferation and excessive formation of syncytial knots.

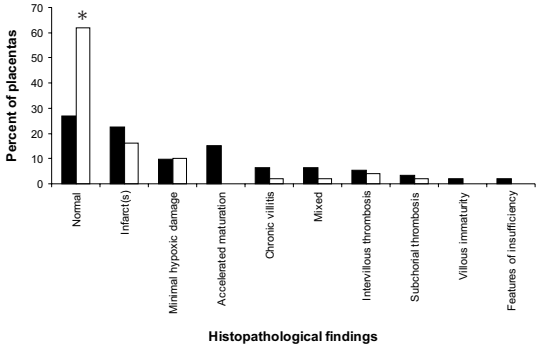

Figure 1. Histopathological findings of placentas from pregnancies complicated by preeclampsia/eclampsia (closed bars, n=279) in comparison to the findings in the group of placentas from uncomplicated pregnancies (open bars, n=50). Asterisk indicates $P<0.001$.

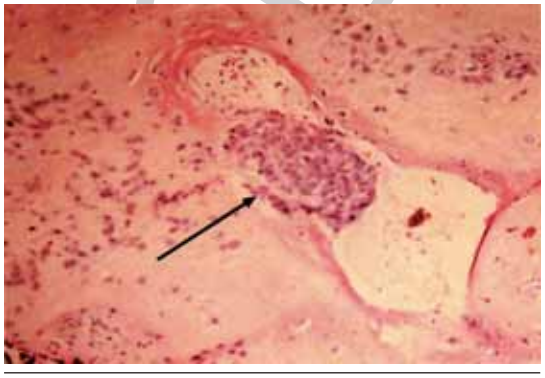

Figure 2. Endovascular trophoblastic plug (arrow) attached to the vessel wall in the basal plate of the third trimester placenta (hematoxylin-eosin, ×100 magnificatin).

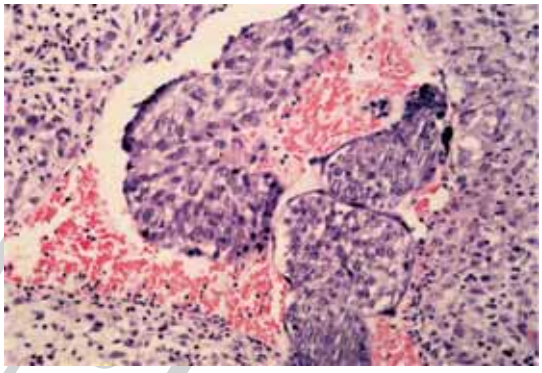

Figure 3. Endovascular trophoblast free in the vessel lumen in the basal plate of the third trimester placenta (hematoxylin-eosin, ×100 magnificatin).

Discussion

The relative frequency of hypoxic changes and acute atherosis in the third trimester placentas from pregnancies complicated by preeclampsia/eclampsia was expected. However, we were surprised not to find significant difference between the appearance of pathological, primarily ischemic changes in the investigated and in the control group, especially since findings of more frequent ischemic changes in placentas from pregnancies complicated by preeclampsia/eclampsia have been published before (6,11). Perhaps the explanation may be due to the fact that about a quarter of the cases in this study were complicated not only by hypertensive disorders but also by diabetes mellitus. Placentas from diabetic pregnancies are characterized by villous immaturity, chorangiosis, and fibrinoid necrosis rather than ischemic changes, but if diabetes was appropriately controlled and treated during pregnancy, the findings may also be normal (6,12). We also found no significant difference in placental weight between the investigated and the control group. Rolled up strips of the placental membranes proved to be a valuable source of decidua vera containing portions of spiral arteries which may show acute atherosis. This was confirmed in this study, because in 3 out of 8 cases of diagnosed acute atherosis, these changes were found in the decidual

layer of the membranes. The latter was histopathologically characterized by fibrinoid necrosis, perivascular mononuclear infiltrate, and infiltration by lipophages. The etiology and pathogenesis of acute atherosis are still unclear, but its similarity to vascular lesions in allograft rejection reaction according to some authors suggests the involvement of the immune system (13,14). 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.

The most unexpected findings in this study were the endovascular trophoblastic plugs in the vessels of the basal plate of placentas from the third trimester of pregnancy. To the best of our knowledge and scarce data from the literature, these plugs should have disappeared by the third trimester (6,15). Currently, there are three hypotheses that try to explain the formation of endovascular plugs. The first, known as the extravasation hypothesis, suggests that endovascular trophoblast derived from an unknown source gains access to the arterial lumens via or close to their point of confluence with the intervillous space and then migrates along the arterial lumens, retrograde to blood flow, by adhering to and replacing endothelium, locally forming intraluminal trophoblastic plugs; some of these cells leave the lumen and centrifugally invade media and adventitia (16). However, the study that proposes this hypothesis was based only on rhesus monkey placentas (16). The second, known as intravasation hypothesis, is based on studies of human placentas and favors the concept according to which the endovascular trophoblast represents an end stage of differentiation of interstitial trophoblast whose subpopulation invades the arterial wall from the outside (17,18). The third hypothesis, by Kam et al (19), is the combination of the two. In any case, the trophoblast that invades arterial walls and forms endovascular plugs originates from the cell columns that connect anchoring villi to the basal plate (20). In the rhesus monkey, the endovascular trophoblast was shown to maintain proliferation, representing a self replicating population in those stages of pregnancy when trophoblastic shell no longer exists (21). Proliferation of endovascular trophoblast in humans has not been observed (22), but we cannot exclude the possibility that it continues until the late stages of pregnancy, contributing to the persistence of endovascular plugs. The expression of cell adhesion molecules is also necessary for trophoblast invasion, because they enable the trophoblast to adhere to the extracellular matrix and target cells in the vessel wall. Some of the studies suggested that trophoblast that approaches the uteroplacental arteries and replaces the endothelium mimicked the adhesion molecule expression pattern found on endothelial cells (23,24). The substantial mass of the extravillous trophoblast follows an interstitial, not an endovascular, pathway of invasion and the maternal cellular environment consisting of decidual cells, endothelial cells, infiltrating macrophages and immune cells. Stromal and vascular smooth muscle cells also influences the trophoblast invasion. There is a gradual shift of integrin expression along the trophoblastic cell columns at the tips of the anchoring villi, switching their attachment preference from basement membrane to stromal matrix substances, as well as changes in the distribution of extracellular matrix components in the uterine wall changes during pregnancy (25-27). Invading trophoblastic cells secrete different metalloproteinases, which play an important role in matrix destruction in association with various cytokines which may act as signaling molecules for local cellular interactions (28,29). The term or near term trophoblast shows diminished attachment capacity on some matrix components *in vitro*, as well as impaired production of proteolytic enzymes (28, 29). Zhou et al (23) showed that the aforementioned integrin shift did not happen in the cytotrophoblastic cell columns in preeclampsia, and suggested that this might be the cellular basis for the disturbed invasion of trophoblast in preeclamptic women. King and Loke (26) showed that maternal endothelial E and P selectin expression occurred only at the implantation site, suggesting a mechanism that enables trophoblast to reside within uteroplacental vessel lumens. Maternal macrophages are also thought to play a role in the regulation of trophoblast invasion and it is shown that activated macrophages induce trophoblast apoptosis *in vitro* (30,31). The morphological findings of endovascular trophoblast in the third trimester placentas strongly indicate that there may be a defect in the expression of certain molecules on the surface of either trophoblastic or vascular endothelial cells. Besides the hypothesis of a diminished expression of certain molecules or

cytokines that cause unsatisfactory physiological change, there could also be overexpression of other adhesion molecules, preventing the endovascular plugs to disengage from the vessel wall and disappear, either by apoptosis or by digestion by macrophages. Although at this moment we do not have a solid proof for this hypothesis, circumstantial evidence speaks in its favor.

Endovascular trophoblastic plugs in placentas from the late second and the third trimester of gestation have been, to the best of our knowledge, mentioned only in the study by Khong et al (9) as an incidental finding. They found endovascular trophoblast in 8 out of 29 (27.6%) placentas from pregnancies complicated by preeclampsia and small for gestational age infants either free in the lumen or more often attached to the intima. The gestation ranged from 28 to 38 weeks, but it is not clear whether all these findings were in the samples from the placental bed (sampled after caesarean hysterectomy in 2 cases of preeclamptic and 7 cases of normal pregnancies, and biopsied in 94 cases from normal and abnormal pregnancies) or some of them were in the placental bed plate from the delivered placenta (sampled in 69 cases). The study of Khong et al, offered no explanation for this phenomenon (9). Our series revealed endovascular plugs in only 1.4% placentas. In comparison to the findings of Khong et al (9), this percentage was strikingly lower. This difference could be explained by the fact that they examined more samples from placental bed biopsy than from the whole placentas, and in their work did not clarify whether the plugs were found in placental bed biopsies or in the basal plate of the entire placentas, whereas we examined the placentas routinely, taking the routine number of samples from a relatively large area of the whole basal plate. In this way, we could have missed a certain number of plugs.

In conclusion, this study showed that in some placentas from pregnancies complicated by preeclampsia and eclampsia, the endovascular trophoblastic plugs remained until the third trimester of pregnancy, possibly contributing to the ischemic damage of placental tissue. On the other hand, there is a possibility that endovascular trophoblastic plugs do not contribute significantly to ischemic damage of the placenta in pregnancy complicated by preeclampsia/eclampsia, but simply reflect disregulated cell to cell interactions in this pregnancy disorder.

References

1 Brosens I, Robertson WB, Dixon HG. The physiological response of the vessels of the placental bed to normal pregnancy. J Pathol Bacteriol. 1967;93:569-79.

2 Robertson WB, Brosens I, Dixon G. Uteroplacental vascular pathology. Eur J Obstet Gynecol Reprod Biol. 1975;5:47-65.

3 Pijnenborg R, Bland JM, Robertson WB, Brosens I. Uteroplacental arterial changes related to interstitial trophoblast migration in early human pregnancy. Placenta. 1983;4:397-413.

4 Pijnenborg R, Bland JM, Robertson WB, Dixon G, Brosens I. The pattern of interstitial trophoblastic invasion of the myometrium in early human pregnancy. Placenta. 1981;2:303-16.

5 Jauniaux E, Jurkovic D, Campbell S. In vivo investigations of the anatomy and the physiology of early human placental circulations. Ultrasound Obstet Gynecol. 1991;1:435-45.

6 Benirschke K, Kaufmann P. Pathology of the human placenta. 2nd ed. New York: Springer Verlag; 1990.

7 Robertson WB, Khong TY, Brosens I, De Wolf F, Sheppard BL, Bonnar J. The placental bed biopsy: review from three European centers. Am J Obstet Gynecol. 1986;155:401-12.

8 Khong TY. Acute atherosis in pregnancies complicated by hypertension, small-for-gestational-age infants, and diabetes mellitus. Arch Pathol Lab Med. 1991;115: 722-5.

9 Khong TY, De Wolf F, Robertson WB, Brosens I. Inadequate maternal vascular response to placentation in pregnancies complicated by pre-eclampsia and by small-for-gestational age infants. Br J Obstet Gynaecol. 1986;93:1049-59.

10 Stallmach T, Hebisch G. Placental pathology: its impact on explaining prenatal and perinatal death. Virchows Arch. 2004;445:9-16.

11 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;189:1173-7.

12 Makhseed MA, Ahmed MA, Musini VM. Impaired gestational glucose tolerance. Its effect on placental pathology. Saudi Med J. 2004;25:1241-4.

13 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;434:207-11.

14 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.

15 Salafia CM, Pijnenborg R. Disorders of the decidua and maternal vasculature. In: Lewis SH, Perrin E, editors. Pathology of the placenta. 2nd ed. New York: Churchill Livingstone; 1999. p. 185-212.

16 Blankenship TN, Enders AC, King BF. Trophoblastic invasion and the development of uteroplacental arteries in the macaque: immunohistochemical localization of

cytokeratins, desmin, type IV collagen, laminin, and fibronectin. Cell Tissue Res. 1993;272:227-36.
17 Damsky CH, Fitzgerald ML, Fisher SJ. Distribution patterns of extracellular matrix components and adhesion receptors are intricately modulated during first trimester cytotrophoblast differentiation along the invasive pathway, in vivo. J Clin Invest. 1992;89:210-22.
18 Fisher SJ, Damsky CH. Human cytotrophoblast invasion. Semin Cell Biol. 1993;4:183-8.
19 Kam EP, Gardner L, Loke YW, King A. The role of trophoblast in the physiological change in decidual spiral arteries. Hum Reprod. 1999;14:2131-8.
20 Kaufmann P, Black S, Huppertz B. Endovascular trophoblast invasion: implications for the pathogenesis of intrauterine growth retardation and preeclampsia. Biol Reprod. 2003;69:1-7.
21 King BF, Blankenship TN. Expression of proliferating cell nuclear antigen (PCNA) in developing macaque placentas. Placenta. 1993;14:A36.
22 Kaufmann P, Castellucci M. Extravillous trophoblast in the human placenta. Trophoblast Research. 1997;10: 21-65.
23 Zhou Y, Fisher SJ, Janatpour M, Genbacev O, Dejana E, Wheelock M, et al. Human cytotrophoblasts adopt a vascular phenotype as they differentiate. A strategy for successful endovascular invasion? J Clin Invest. 1997; 99:2139-51.
24 Zhou Y, Damsky CH, Fisher SJ. Preeclampsia is associated with failure of human cytotrophoblasts to mimic a vascular adhesion phenotype. One cause of defective endovascular invasion in this syndrome? J Clin Invest. 1997;99:2152-64.
25 Burrows TD, King A, Loke YW. Trophoblast migration during human placental implantation. Hum Reprod Update. 1996;2:307-21.
26 Burrows TD, King A, Loke YW. Expression of adhesion molecules by endovascular trophoblast and decidual endothelial cells: implications for vascular invasion during implantation. Placenta. 1994;15:21-33.
27 Burrows TD, King A, Smith SK, Loke YW. Human trophoblast adhesion to matrix proteins: inhibition and signal transduction. Hum Reprod. 1995;10:2489-500.
28 Campbell S, Rowe J, Jackson CJ, Gallery ED. In vitro migration of cytotrophoblasts through a decidual endothelial cell monolayer: the role of matrix metalloproteinases. Placenta. 2003;24:306-15.
29 Campbell S, Rowe J, Jackson CJ, Gallery ED. Interaction of cocultured decidual endothelial cells and cytotrophoblasts in preeclampsia. Biol Reprod. 2004;71: 244-52.
30 Hunt JS. Current topic: the role of macrophages in the uterine response to pregnancy. Placenta. 1990;11:467-75.
31 Reister F, Frank HG, Kingdom JC, Heyl W, Kaufmann P, Rath W, et al. Macrophage-induced apoptosis limits endovascular trophoblast invasion in the uterine wall of preeclamptic women. Lab Invest. 2001;81:1143-52.

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