Spontaneous Abortion and Failure of Human Cytotrophoblasts to adopt a vascular adhesion phenotype

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Summary:

Background: *Differentiating cytotrophoblasts transform their adhesion receptor phonotype so as to resemble the endothelial cells they replace a process that is required for normal placentation, and any defect in this adhesion phenotype switch might lead to pregnancy failure.*
Patients and Methods: *Curate samples of the materno-fetal interface were taken from 20 women with spontaneous first trimester abortion and 6 control women undergone elective termination of pregnancy in the first trimester. Immunohistochemistry analysis of paraffin embedded sections of these curate samples was performed using monoclonal antibodies for cytokeratin, PECAM-1 and VCAM-1.*
Results: *Cytokeratin showed positive immunostaing of the cytotrophoblasts lining the blood vessels, and PECAM-1 was positive in the cytotrophoblasts in only two cases who had elective termination of their pregnancy, while VCAM-1 immunostaining of the cytotrophoblasts was positive in three cases that had elective termination of pregnancy and only two cases that had spontaneous abortion.*
Conclusion: *Defective expression of endothelial cell adhesion molecules in human cytotrophoblasts might predispose to abnormal placentation, pregnancy failure and subsequent abortion.*
Key wards: *Abortion.. cytotrophoblasts, PECAM-1, VCAM-1.*

Introduction:

Establishment of the human placenta requires that fetal cytotrophoblasts (CTB) in the anchoring chorionic villi become invasive. These CTB aggregate into cell columns and invade both the uterine interstitium and vasculature, anchoring the fetus to the mother and establishing blood flow to the placenta. CTB colonize the maternal vasculature and replace the endothelium as far as the first inner third of the myometrium and differentiating CTB transform their adhesion receptor phenotype so as to resemble the endothelial cells they replace (1,2,3). The interaction of the trophoblasts with components of the vascular compartment, and particularly with the endothelium, are complex and require the upregulation of specific adhesion molecules capable of mediating this heterotypic binding. The molecular aspects of this interaction are still poorly characterized (4),

Failure .of trophoblast invasion and spiral artery transformation has been documented in preeclampsia, in which reduced uteroplacental perfusion is associated with widespread endothelial dysfunction and fetal growth retardation (5,6).

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Similar vascular abnormalities has been reported in the placental bed of women with fetal growth retardation and spontaneous abortion in the absence of maternal hypertension (7,8,9,10). Thus failure of the spiral arteries to undergo physiological transformation may lead to spectrum of pregnancy failures. Despite the importance of trophoblast invasion and vascular remodeling, these processes are still not well understood. However, they are thought to include changes in expression of cell adhesion molecules, matrix metalloproteinases and their tissue inhibitors, and growth factors and their receptors (11,12).

Platelet endothelial cell adhesion molecule PECAM-1 or CD31 is about 130 kd transmembrane glycoprotein and a member of the immunoglobulin family (13,14). It is present on endothelial cells, platelets, monocytes and lymphocytes. Its expression begins early in development and persists through adulthood (15). During development, PECAM-1 is expressed early in the blastocyst, in the pre-somite embryo in angioblasts and in yolk-sac blood islands and expression persist throughout embryonic development (16). PECAM-1 is localized to cell-cell borders of adjacent endothelial cells suggesting a role in angiogenesis (17). Also PECAM-1 and other endothelial cell adhesion molecules (CAMs) may play a role in decidual spiral artery transformation (3,18).

Vascular cell adhesion molecule VCAM-1 was originally identified as a cytokine inducible surface protein that mediate adhesion of a number of

leukocytes including lymphocytes, monocytes, mast cells, eosinophils, and tumor cells to umbilical vein endothelial cells (19,20). VCAM-1 function may also be important in embryonic development, VCAM-I-defcient mouse embryos displayed two distinct phenotypes; one group of embryos failed to develop a functional extra-embryonic circulation due to failure of the allantois to fuse with the chorion, the other group displayed several defects in the developing heart, suggesting that they may have subsequently died from abnormal cardiac function (21,22).

In this study we tried to localize these two adhesion molecules, PECAM-1 and VCAM-I in the trophoblasts of women with spontaneous abortion and women with normal pregnancy undergone elective termination in the first trimester, to find out whether there is defective expression of these adhesion molecules in the trophoblasts of women with spontaneous abortion. In addition, cytokeratin immunostaining was carried out in order to assist in the identification of the trophoblasts (3).

Materials and methods

Patients and controls

Samples were obtained from women at Al-Kadlunyia teaching hospital-Baghdad-Iraq from October 2003 to April 2004. Patients were 20 women with spontaneous abortion in the first trimester, undergone evacuation curate operation, while the controls were 6 women undergone elective termination of apparently normal pregnancy for a maternal medical indication under approved consent of two senior gynecologists and a physician. Samples were taken after evacuating the uterine cavity, fixed in 10% buffered formalin overnight, and were subsequently embedded in paraffin.

Immunohistochemistry

5µm thickness tissue sections were obtained for Immunohistochemistry staining. Immunohistochemistry was performed using DakoCytomation LSAB2 System-HRP code K0673 (DakoCytomation, USA), Immunohistochemistry detection kit, contains: Peroxidase Block (3% hydrogen peroxide in water), Biotinylated Link (biotin labeled goat anti-rabbit and goat anti-mouse inununoglobulins), Strepavidin-HRP (strepavidin conjugated to horseradish peroxidase), and Substrate buffer, DAB Chromogen (3,3'-diaminobenzidine in a chromogen solution). The monoclonal antibodies are listed in the table.

Monoclonal antibodies included in the study

Antibody type	Dilution	Source	Specificity
Cytokeratin	1:100	BioGenex USA	Human cytokeratin 7 (CK7)
PECAM-1	1:40	DAKO Denmark	The extracellular domain of CD31 molecule
VCAM-1	1:50	DAKO Denmark	Antibody clustered as anti-CD 106 molecule

Tissue sections were deparaffinized in xylene for 5 minutes and rehydrated through a series of ethanol dilutions, after retrieval of the tissue antigen in a citrate buffer under 121 ℃ in the autoclave for two minutes.. the sections were washed in distilled water, and then endogenous peroxidase was blocked using peroxidase block for 30 minutes (23), 100µl of the diluted primary (monoclonal) antibody was applied onto the sections and incubated overnight at 37°C, then washed in a phosphate buffer saline (PBS) bath for 5 minutes, followed by applying the biotinylated link (secondary antibody) and incubation at 37°C for one hour, and then the same washing in PBS was performed, followed by placing 1-2 drops of the Strepavidin-HRP conjugate onto the tissue sections and incubation at 37°C for one hour then washing, and then the DAB substrate chromogen was applied for 10-20 minutes yielding a brown signal at the antigenic site, followed by counterstaining with Mayer's hematoxylin.

Negative controls were obtained by omitting the monoclonal antibody and using antibody diluent alone to verify the signal specificity. Human tonsilar tissue was used as a positive control tissue for both PECAM-1 and VCAM-1 immunostaining. Positive cells displayed staining of the cell membrane with weaker cytoplasmic staining (as instructed by the manufacturer).

Results:

Cytokeratin showed obvious staining of the villus cytotrophoblasts (fig 1, A and B), and interstitial CTB (fig 1, C) with obvious membranous staining (fig 1, D). In addition cytokeratin stained cytotrophoblast cells around and lining the blood vessels but not in the lumen (fig 2), decidual cells and decidual blood vessels were also positive for cytokeratin immunostaining (fig 2, D).

PECAM-1 (CD31) immunostaining of the trophoblasts was positive in only 2/6 (33.3%) of women with elective termination of pregnancy in the first trimester (who were 12 weeks gestational age) (fig 3, A and B), while the trophoblasts in all the women who had spontaneous abortion were negative for PECAM-1 immunostaining.

VCAM-1 (CD106) showed positive immunostaining of the trophoblasts in 3/6 (50%) of women with elective termination of pregnancy and in only 2/20 (10%) of women with spontaneous abortion at 14 weeks gestational age (fig 3, C), with very obvious membranous staining of the cytotrophoblasts (fig 3, D).

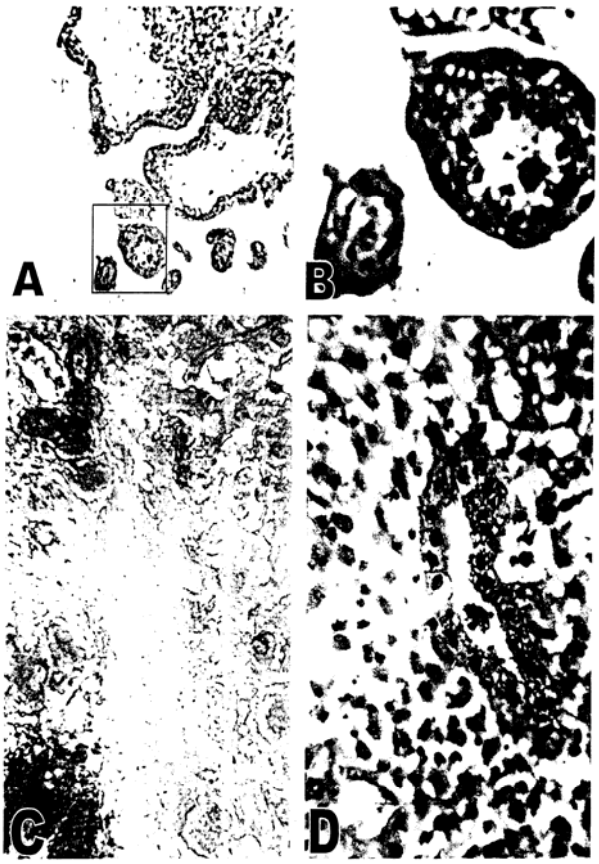

Figure (1) Immunohistochemical staining of Cytokeratin in women with abortion. Staining by DAB chromogen (brown) counterstained with Mayer's heamatoxylin. (**A**) Cytokeratin positive villus trophoblasts. (**B**) Higher magnification of A. (**C**) Cytokeratin positive decidual cells and invasive trophoblasts. (**D**) Membranous staining of the trophoblasts. Magnification power of A (X100), B-D (X400).

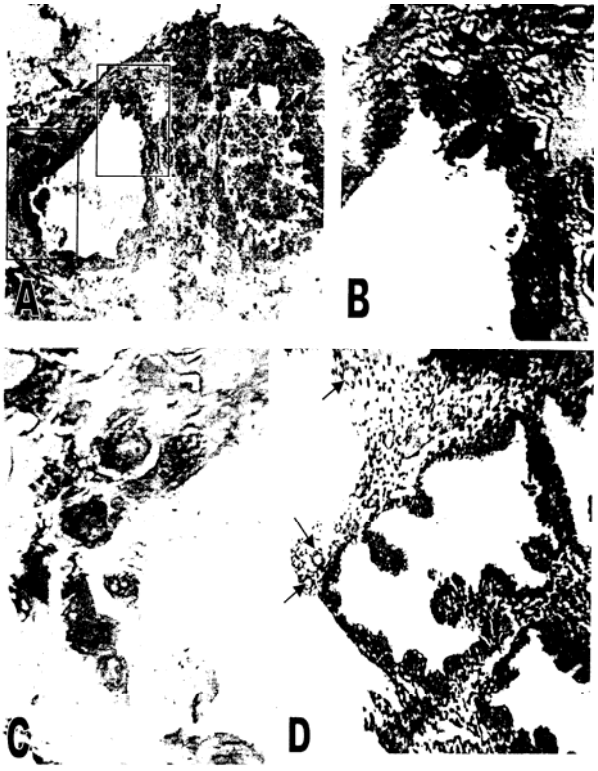

Figure (2) Immunohistochemical staining of Cytokeratin in women with abortion. Staining by DAE chromogen (brown) counterstained with Mayer's heamatoxylin. (A) Cytokeratin positive trophoblasts lining anc around the blood vessels but not in the lumen. (**B**) and (**C**) higher magnification of A demonstrate trophoblasts lining the blood vessel. (**D**) Cytokeratin positive decidual cells and blood vessels (arrows). Magnification power of A and D (X100), B and C (X400).

Figure (3) Immunohistochemical staining of PECAM-1 and VCAM-1 in women with abortion. Staining by DAB chromogen (brown) counterstained with Mayer's heamatoxylin. (**A**) and (**B**) PECAM-1 positive cytotrophoblasts in women with induced abortion. (**C**) VCAM-1 positive cytotrophoblasts in a woman with induced abortion. (**E**) Higher magnification of C. (**D**) VCAM-1 shows membranous staining of the trophoblasts in a woman with spontaneous abortion. Magnification power of A, B, D and E (X400), and C (X100).

Discussion:

Could human trophoblasts adopt a vascular adhesion phenotype?

During the first half of gestation, a subset of chorionic villus cytotrophoblasts leave the fetal compartment and invade the uterine wall and its vascular network, thereby anchoring the fetus to the mother and colonizing the maternal vasculature as far as the first inner third of the myometrium. Cytotrophoblasts replace the maternal endothelium on the vessel wall in this process. In order to accomplish this extraordinary feat, it is likely that this subset of cytotrophoblasts must not only acquire an invasive phenotype (24,25), but must also transform their adhesion molecule phenotype in a comprehensive manner so as to mimic that of cells of the vascular system, particularly endothelial cells (3). Our results support this idea when cytotrophoblasts showed enhanced staining of adhesion molecules characteristic of endothelial cells and certain leukocytes. These include VCAM-1 and PECAM-1, which is in line with the results of other studies (3,4).

To have a more support to our results, cytokeratin staining showed positive invasive cytotrophoblasts and cytotrophoblasts in the

decidual stroma, and demonstrated the presence of cytotrophoblasts in the lining of, and surrounding, the decidual blood vessels, suggesting that these cytotrophoblasts had replaced the endothelial lining of these blood vessels transforming them into larger diameter low resistance vessels, which is in agreement with other studies (3,26,27).

Human trophoblasts invasion occur in two waves; the first into the deciduas at 8-10 weeks of gestation, and the second into the myometrium at 16-18 weeks of gestation and these physiological changes are required for a successful pregnancy, which means that the majority of these vascular changes starts between 10-16 weeks of gestation (27,28), this could explain our results which showed that PECAM-1 and VCAM-1 were expressed in the trophoblasts of women at the gestational ages of 12-14 weeks, i.e. during the period of highest rate of invasiveness of the cytotrophoblasts, and they were negative in the trophoblasts of women at the gestational ages below 10 weeks even in normal pregnant women (27,28).

PECAM-1 is expressed *in vivo* and *in vitro* by human trophoblasts and appears to be involved in the interaction between trophoblasts and endothelial cells (4). In addition PECAM-1 is localized to the cell-cell borders of adjacent endothelial cells suggesting a role in angiogenesis (29), and in support of this notion, about 20-30% of PECAM-1 was found associated with cytoskeleton in confluent endothelial cells increasing to about 65% during cell migration (16). Also PECAM-1 in conjunction with VE-cadherin might regulate processes such as endothelial cell tube formation, and antibodies against PECAM-1 markedly inhibited the ability endothelial cells to organize and form three-dimensional network in matrigel (30). All these evidences support the role of PECAM-1 in angiogenesis, indicating that localization of PECAM-1 in the trophoblasts in normal pregnant women might be important in trophoblasts epithelial-endothelial conversion and formation of new blood vessels. And failure to localize PECAM-1 in women with spontaneous abortion even in those with 14 weeks gestational age, could point to its important role in the maintenance of pregnancy.

On the other hand, VCAM-1 expression on the trophoblasts is also important; not only for blood vessel formation, but also to support adhesion and placentation, and several observations demonstrated that VCAM-1-a41ntegrin interaction is critical for effective placentation (31). These studies support the important role of VCAM-1 in placentation and blood vessel formation. and its defective expression in the majority of women with spontaneous abortion might point to its important role for pregnancy to succeed.

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