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Research Article

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Changes in the Expression of Immunohistochemical Markers of Angiogenesis and Apoptosis in Chorionic Villi during Early Pregnancy Miscarriage

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Abstract

Objective: Aim of this study was to investigate changes in the expression of immunohistochemical markers of angiogenesis (VEGF) and apoptosis (Caspase 3) in chorionic villi of human embryos, obtained after sporadic, recurrent pregnancy miscarriage and compare them with such expression in normal pregnancy Chorionic villie.

Methods: Histological material included 63 recurrent, sporadic miscarriage and 32 artificial abortions chorionic villi samples. Immunohistochemical technique was applied to study expression levels of VEGF and Caspase 3.

Results: VEGF and Caspase 3 expression intensity and diversity in different Chorionic villie (CV) structural components was observed in different investigated groups.

Conclusion: Our research has shown that an important role is played not only by a general decrease in VEGF expression but also a change in the allocation of this cytokine in the structural components of early pregnancy miscarriage CV. The processes of apoptosis were more active in the groups of early miscarriage; however, they did not block the synthesis of VEGF. These results may indicate the role of factors of angiogenesis and apoptosis in the development of early miscarriage of pregnancy.

Keywords

Immuno histochemical markers; Early pregnancy miscarriage; Idiopathic pregnancy

Introduction

Today the issue of pregnancy miscarriage (PM) remains very topical, since the frequency of clinically verified PM remains a major complication of pregnancy. About a quarter of all pregnancies end with PM and in most cases early miscarriage (up to 10-12 weeks of fetal development) is diagnosed [1]. This pathology is divided into two main types – sporadic (SPM, one or two miscarriages) and recurrent (RPM, more than 3 miscarriages) PM. RPM is observed in 2-5% of all clinically verified pregnancies [2]. In about 50% of cases, the cause of recurrent miscarriage is unidentified and is defined as "idiopathic pregnancy miscarriage", which also includes immune infertility since its mechanisms are also not fully studied [3].

One of the main components of the feto-maternal interface is the chorionic villi (CV), which are highly vascularized and capable of active proliferation. CV in the first trimester provides the embryo with oxygen through the microcirculatory system and protects it against the negative effects of various factors of the maternal organism (autoimmune response, bacterial or other invasion, etc.). Versatile study of CV functions, including - angiogenetic, in the normal case and in the case of early miscarriage of pregnancy, will make it possible to understand better the mechanisms of development of this process.

VEGF (Vessel Endothelial Growth Factor) is a multifunctional cytokine that is synthesized by many cells and whose main function is angiogenesis control [4]. VEGF accelerates the proliferation and migration of endothelial cells, slowing the processes of apoptosis, increases vascular permeability and accelerates stromal proteolysis [5]. VEGF is a critical factor that plays one of the key roles in the development of the embryo, its implantation and decidualization [6,7]. Various authors showed the opposite results of their studies on VEGF expression in CV cells obtained after sporadic and habitual miscarriage of pregnancy and were associated with the possible development or absence of apoptosis in chorionic villus components [5,8,9].

One of the central roles in the cascade of reactions, leading to apoptosis of the cell, belongs to the family of caspases, proteases, which are divided into 12 types and which can be divided into two main subgroups – initiators (eg, 1, 8, 9 types) and executor caspases (for example, 3, 6, 7 types) [10-12]. Caspase 3 is a protease that belongs to the executor caspases group and is activated by initiator caspases to trigger mechanisms of apoptosis.

The idiopathic recurrent pregnancy miscarriage from the point of view of the apoptosis of the hematoplacental barrier components was studied by scientists, mainly by determining the expression of apoptosis-dependent genes in chorionic villi or immunohistochemical (IHC) and other studies of decidual tissue. There was a certain association between RPM and excessive expression of apoptotic-dependent genes [13,14].

Aim of this study was to investigate changes in the expression of immunohistochemical markers of angiogenesis (VEGF) and apoptosis (Caspase 3) in chorionic villi of human embryos, obtained after sporadic, recurrent pregnancy miscarriage and compare them with expression in normal pregnancy CV.

Materials and Methods

63 CV tissue samples were obtained after uterine cavity curettage after sporadic, recurrent miscarriages and 32 samples after artificial abortions, at women requests, from 5 to 12 weeks of gestation, confirmed by an ultrasound scan and related medical documentation. The group of sporadic miscarriage included 38 CV samples from women who already have children and applied for medical assistance for the first or second PM in their lives. The recurrent miscarriage group included 35 CV samples from women who had applied for

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medical assistance more than 3 times and did not have children. The excluding factors from this group were as follows: the diagnosis of antiphospholipid syndrome, autoimmune thyroiditis, SLE, rheumatoid polyarthritis or sclerodermia in women and chromosomal abnormalities of the fetus, confirmed by the cytogenetic method. The control group (CG) included 32 CV samples from women who had children and who had undergone an artificial abortion procedure at their request. Exceptional factors were the following: the absence of children and the chromosomal abnormalities of the fetus, diagnosed with cytogenetic method.

From the obtained material, chorionic villi were obtained, washed with 0.9% NaCl solution and fixed in Buen solution. After this – the forming of histological material paraplast blocks was carried out according to the standard scheme [15].

Mayer's hematoxylin and eosin staining was performed according to standard technique [3].

Histologic sections, 4-6 μm thick, were applied to the SuperFrost Plus adhesive slides. After deparaffinization and rehydration of the sections, temperature demasking of antigens was performed – heat induction of epitope retrieval (sections were placed in citrate buffer with pH 6.0 and heated in an autoclave at a temperature of + 121°C for 8 minutes) and suppressed the activity of endogenous peroxidase with a 3% solution of hydrogen peroxide for 20 minutes. Subsequently, incubation of sections with primary antibodies in moist chambers at a temperature of 230-250°C for 30 minutes was performed. As primary, monoclonal antibodies to Caspase 3 and VEGF (TermoScientific, USA) (Table 1) were used. The titre of antibodies was selected according to the manufacturer's recommendations, using the special solvent Antibody Diluent (TermoScientific, USA). To identify the reaction, Quanto's visualization system (TermoScientific, USA) was used, with the application of 3-diaminobenzidine tetrachloride (DAB) as chromogen (TermoScientific, USA). To separate non-colored structures, the sections were further treated with Mayer's hematoxylin.

The study was conducted with the permission of women on the histological material, fixed and directed to the Institute of Hereditary Pathology of the National Academy of Medical Sciences of Ukraine for cytogenetic analysis from September 2016 to August 2017 and was carried out in accordance with the main standards of the GCP (1996), the European Convention on Human Rights and Biomedicine 04.04.1997, Helsinki Declaration of the World Medical Association on the Ethical Principles of Scientific Medical Research with Involvement of People (1964-2008), Order of the Ministry of Health of Ukraine No. 690 dated September 23, 2009 and in agreement with the Bioethics Commission of Danylo Halytsky Lviv National Medical University (Protocol No. 2 dated February 15, 2016).

Results

Stained with hematoxylin and eosin chorionic villi included following components: syncytiotrophoblast (STB) with microvilli on its outer surface, cytotrophoblast (CTB), mesenchyme (MC), which includes fibroblasts, connective tissue fibers, Hofbauer cells (HC) and vessels of the microcirculatory system (Figure 1).

In 10 out of 35 (28%) CV samples of the RM group, there was a detachment and consolidation of mesenchyme, thinning of STB and CTB, which can be characterized as destructive tissue changes. A similar pattern was observed in less CV samples from the SM group (in 5 samples (13%)). In the CV control group, such changes were not noted in any case (Figure 1). It should also be noted that the number of vascular components of MC in the first two groups was also lower, especially in chorionic villi with dystrophic changes.

VEGF

A high expression of VEGF in the cytoplasm of the CTB cells and vascular endothelium was observed in the control group CV tissues. In 12% of cases, in addition to the aforementioned components, there was also high expression in the cytoplasm of STB and on its outer surface, namely, in microvilli.

In the group of sporadic miscarriage, a moderate expression of the aforementioned cytokine in the cytoplasm of the cells of the CTB, the syncytial layer and the endothelium of the vessels, and absent – in all other cells of the mesenchyme was established. In other words, the exposure to VEGF was evenly distributed in the coating layers of CV.

The distribution of VEGF expression sites in the histological material of the RM CV was similar to the previous group (Figure 2,A,C,E).

Caspase 3

In the control group, high expression was observed in the cytoplasm of the CTB cells, vascular endothelium, and fibroblasts. In 12% of cases, as with the use of antibodies to VEGF, high expression of caspase 3 was observed in the STB cytoplasm and on its outer surface.

In CV tissues of sporadic and recurrent miscarriage groups, a high expression of this proteinase in the cytoplasm of STB, CTB and moderate in the endothelium of blood vessels and fibroblasts was observed (Figure 2,B,D,F).

The summarized results are presented in Table 2 and Figure 3.

Conclusion

Chai et al. and He et al. in their studies showed a decrease in VEGF expression in early spontaneous and recurrent miscarriage of pregnancy [5,8]. Pang et al. on the contrary, showed increased VEGF expression in CV components during RM and linked the difference between their and previous observations to the possible delay in few days between pregnancy loss and collecting of the CV material, which may lead to apoptotic changes and, correspondingly, defects in VEGF expression in structural components of CV [9].

Our research has shown that an important role is played by not only a general decrease in VEGF expression but also a change in the allocation of this cytokine in the structural components of CV. A similar situation was observed with the expression of Caspase 3. Thus, Syncytiotrophoblast is a component that first reacts to the disorder of the hematoplacental barrier including the development of apoptosis and the onset of the synthesis of VEGF.

In our opinion, the change in the distribution of VEGF and Caspase 3 expression may be due to the development of compensatory responses to adverse maternal factors (e.g., the immune response to the embryo).

It should also be noted that the processes of apoptosis were indeed more active in the groups of early miscarriage; however, they did not block the synthesis of VEGF.

Table 1: Panel of primary antibodies.

Antibody	*Clone*	*Titre*	*Manufacturer*
Caspase 3	Clone 31A893	1:50	TermoScientific
VEGF	Clone VG1	1:100	TermoScientific

To evaluate the IHC reaction, the intensity of the color was evaluated in 4 categories (0, +, ++, +++), where 0 – no staining, + – weak, ++ – moderate and +++ – intensive staining.

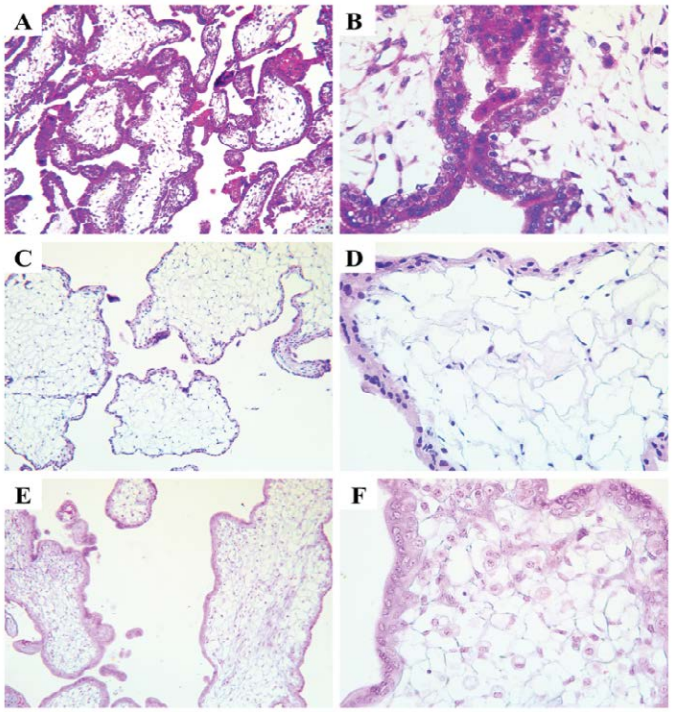

Figure 1: Chorionic villi of the human embryos obtained after an early pregnancy miscarriage and artificial abortions, stained with hematoxylin and eosin. **A, B** – chorionic villi of the control group; **C, D** – chorionic villi of a group of sporadic miscarriage; **E, F** – chorionic villi of the recurrent miscarriage group.

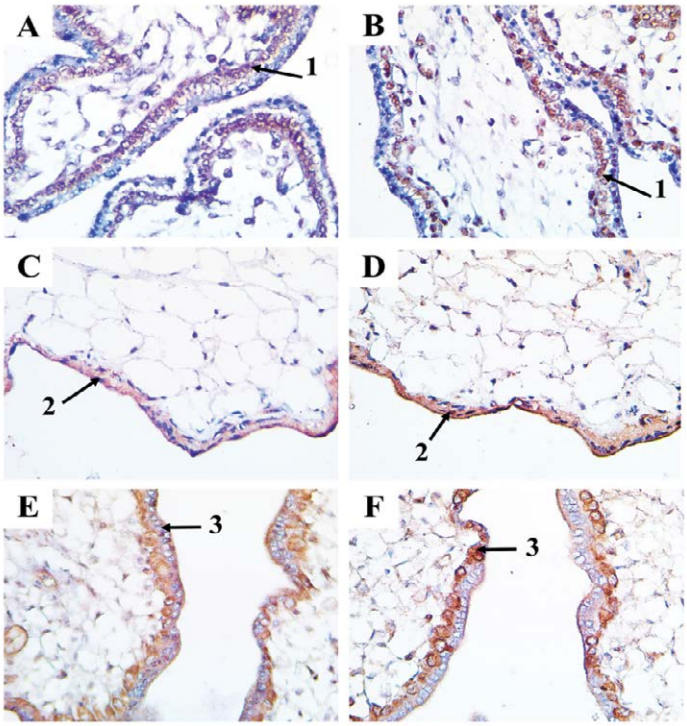

Figure 2: Expression of VEGF (A, C, E) and Caspase 3 (B, D, F) in the structural components of the chorionic villi of the human embryos obtained after an early pregnancy miscarriage and artificial abortions (x400).

A, B – chorionic villi of the control group (1 – high expression of markers in cytotrophoblast); **C, D** – chorionic villi of a group of sporadic miscarriage (2 – expression of markers in cytotrophoblast and syncytiotrophoblast); **E, F** – chorionic villi of the recurrent miscarriage group (3 – expression of markers in cytotrophoblast and syncytiotrophoblast).

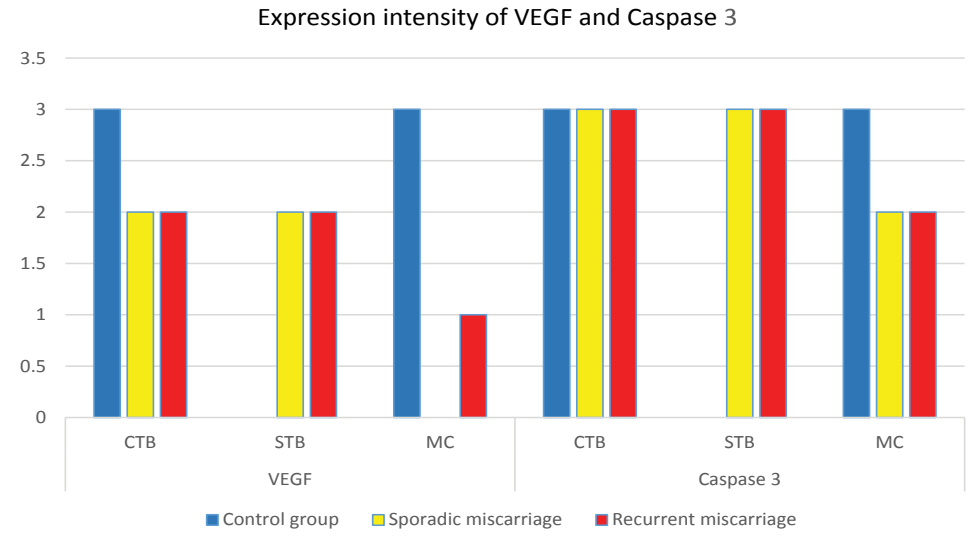

Figure 3: Expression intensity of VEGF and Caspase 3.

CTB – cytotrophoblast, STB – syncytiotrophoblast, MC – mesenchyme

Table 2: Expression intensity of VEGF and Caspase 3.

Components \ Group		Control group	Sporadic miscarriage	Recurrent miscarriage
VEGF	Cytotrophoblast	+++*	++	++
	Syncytiotrophoblast	-	++	++
	Mesenchyme	+++	-	+
Caspase 3	Cytotrophoblast	+++	+++	+++
	Syncytiotrophoblast	-	+++	+++
	Mesenchyme	+++	++	++

* – "-" – no staining, "+" – weak staining, "++" – moderate staining, "+++" – intensive staining

These results may indicate the role of factors of angiogenesis and apoptosis in the development of early miscarriage of pregnancy.

References

1. Kolte AM, Bernardi LA, Christiansen OB, Quenby S, Farquharson RG, et al. (2014) Terminology for pregnancy loss prior to viability: a consensus statement from the ESHRE early pregnancy special interest group. Human Reproduction 30: 495-498.
2. Ware BD, Gibson M, Silver RM (2010) Recurrent miscarriage. N Engl J Med 363: 1740-1747.
3. Avwioro G (2011) Histochemical uses of haematoxylin-a review. Jpcs 1: 24-34.
4. McKeeman GC, Ardill JE, Caldwell CM, Hunter AJ, McClure N (2004) Soluble vascular endothelial growth factor receptor-1 (sFlt-1) is increased throughout gestation in patients who have preeclampsia develop. Am J Obstet Gynecol 191: 1240-1246.
5. He X, Chen Q (2016) Reduced expressions of connexin 43 and VEGF in the first-trimester tissues from women with recurrent pregnancy loss. Reprod Biol Endocrinol 14: 46.
6. Zygmunt M, Herr F, Münstedt K, Lang U, Liang OD (2003) Angiogenesis and vasculogenesis in pregnancy. Eur J Obstet Gynecol Reprod Biol 110: S10-S18.
7. Eller AG, Branch DW, Nelson L, Porter TF, Silver RM (2011) Vascular endothelial growth factor – a gene polymorphisms in women with recurrent pregnancy loss. J Reprod Immunol 88: 48-52.
8. Chai L, Ling K, He X, Yang R (2013) Expression of ATF4 and VEGF in chorionic villus tissue in early spontaneous abortion. Eur J Obstet Gynecol Reprod Biol 170: 434-438.
9. Pang L, Wei Z, Li O, Huang R, Qin J, et al. (2013) An increase in vascular endothelial growth factor (VEGF) and VEGF soluble receptor-1 (sFlt-1) are associated with early recurrent spontaneous abortion. PloS one 8: e75759.
10. Lamkanfi M, Declercq W, Kalai M, Saelens X, Vandenabeele P (2002) Alice in caspase land. A phylogenetic analysis of caspases from worm to man. Cell death and differentiation 9: 358-361.
11. Saleh M, Vaillancourt JP, Graham RK, Huyck M, Srinivasula SM, et al. (2004) Differential modulation of endotoxin responsiveness by human caspase-12 polymorphisms. Nature 429: 75-79.
12. Boatright KM, Salvesen GS (2003) Mechanisms of caspase activation. Curr Opin Cell Biol 15: 725-731.
13. Choi HK, Choi BC, Lee SH, Kim JW, Cha KY, et al. (2003) Expression of angiogenesis-and apoptosis-related genes in chorionic villi derived from recurrent pregnancy loss patients. Mol Reprod Dev 66: 24-31.
14. Zhao W, Shen WW, Cao XM, Ding WY, Yan LP, et al. (2017) Novel mechanism of miRNA-365-regulated trophoblast apoptosis in recurrent miscarriage. J Cell Mol Med 21: 2412-2425.
15. Antonyuk VO (2005) Lectins and their material sources, Lviv.

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