

Association of Growth Factors Genes with Miscarriage

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Abstract

Background: The study was aimed to investigate the association of *VEGFA* gene polymorphic variants -2578C>A (rs699947) and -634G>C (rs2010963) and *TGFBI* gene 915G>C (rs1800471) and gene expression level with miscarriage in the first trimester.

Methods: 288 women with different courses of pregnancy and 61 chorionic tissue samples were involved in case-control study. Allele-specific polymerase chain reaction in real time was used for genotyping. Next, gene-gene interactions were analyzed using the multifactor dimensionality reduction method. *VEGFA* and *TGFBI* genes expression levels were determined by RT-PCR.

Results: It was found that SNP rs699947 was associated with the miscarriage risk change ($p=0.05$). The *CC* genotype was associated with reduced risk of abortion in the first trimester, and the *CA* genotype with increased risk. Genotypes *VEGFA* -2578CC/*VEGFA* -634CG, *VEGFA* -2578AA/*VEGFA* -634CG, and *VEGFA* -2578CC/*VEGFA* -634CG/*TGFBI* 936CC were associated with lowered risk of miscarriage in the first trimester. mRNA level of *TGFBI* was significantly higher in decidual tissue compared to chorionic tissue in normally progressing pregnancy ($p=0.003$). *VEGFA* gene expression level was directly correlated with the *TGFBI* mRNA level ($R=0.60$; $p=0.038$). In pregnancy loss, an inverse relationship was observed ($R=-0.76$; $p=0.028$).

Conclusion: The SNP rs699947 is associated with pregnancy loss in the first trimester. The MDR analysis data showed the significant relationship between *VEGFA* and *TGFBI* genes in two-locus and three-locus models. A change in the ratio of the concentrations of growth factors can disrupt the processes of cell division, apoptosis and angiogenesis processes.

Keywords: Chorionic tissue, Decidua, Gene expression, Gene polymorphic variants, Growth factors, Miscarriage.

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Introduction

Growth factors are the signal protein molecules stimulating the cell growth, proliferation, regulation and interaction. Growth factors play a key role in proliferation control and differentiation of different cell types, specific immunity activation, inflammation regulation, angiogenesis, neuronal functioning, tissue regeneration and embryonic development (1-4).

Vascular endothelial growth factor (VEGF) is one of the key factors in the capillaries formation.

VEGFA is produced in almost all tissues and increases vascular permeability and endothelial cell proliferation (5-7). *TGFBI* protrudes as the antagonist of VEGF in cell proliferation and induction of apoptosis (8). *TGFBI* promotes wound repair processes, inhibits smooth muscle and endothelial cells proliferation and migration. Almost every cell in the body produces *TGFBI* and its receptor (9). However, *TGFBI* has inhibitory effects on the immune system, hematopoiesis, the synthe-

sis of pro-inflammatory cytokines, the response of lymphocytes to IL2, IL4, IL7 and the formation of cytotoxic NK and T cells (9-11).

It has been shown that TGFB1 is secreted by blastocyst cells (12). Subsequently, decidual stromal cells and NK cells in turn produce TGFB, which is necessary for the trophoblast growth and development (13). The first signs of placental angiogenesis are observed at the third week of pregnancy (14). Cytotrophoblast cells intensely express chorionic gonadotropin, which influences angiogenesis and expression of endothelial vascular growth factor positively (15). Spiral arteries remodeling is crucial for normal fetus growth and development. Violations of current implantation stages and fetal-maternal blood formation can usually lead to pregnancy loss.

Among the known *TGFB* gene polymorphisms, two SNPs 869T>C and 915G>C in exon 1 result in amino acid substitutions in codon 10 (Leu-Pro) and codon 25 (Arg-Pro), respectively (16). In the *VEGFA* gene, four polymorphic sites are widely studied, i.e. -2578C>A, -1154G>A, -634G>C, 936C>T, which are associated with a recurrent pregnancy loss (17, 18). However, the clinical significance of *VEGFA* and *TGFB* gene polymorphisms has not been fully proven and the data are contradictory (2, 15, 18-21). Literature data on allele frequencies and genotypes of *VEGFA* and *TGFB* SNPs in chorionic tissue in miscarriage in the first trimester is absent. Also, growth factors gene-gene interactions studies which are associated with a recurrent pregnancy loss in the first trimester are few.

The current research was conducted to investigate the association of *VEGFA* gene polymorphic variants -2578C>A (rs699947) and -634G>C (rs2010963) and *TGFB* gene 915G>C (rs1800471) and gene expression level with miscarriage in the first trimester.

Methods

Description of studied groups: A total of 288 wom-

en were analyzed. Control group included 145 women with physiological pregnancy without a history of spontaneous abortion and was compared with 143 women with miscarriage in 5-11 weeks of pregnancy. The average age of women in both groups was 29.5 years.

All procedures performed in studies were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The participants willingly signed the informed consent. For the formation adequate groups, a questionnaire was prepared, including questions about anamnesis, objective status and environmental conditions of the women. Women with uterine abnormalities and polycystic ovary syndrome, previously diagnosed with arterial hypertension, diabetes, thyroid disease and autoimmune pathology, as well as infectious diseases during pregnancy, were excluded from the study. Women with exogenous risk factors, such as alcohol abuse, exposure to harmful professional factors (E.g., electromagnetic radiation, noise, vibration, chemical production) were excluded on the result of the questioning. BMI was in normal range of 23.9±5.1 for control group and 23.4±3.37 for women with miscarriage.

Blood samples and anamnesis were collected and the investigated groups were formed in the maternity hospital No. 5, the obstetrician-gynecologists of city hospital No. 8 and the Center for Reproduction and IVF in Rostov-on-Don. The majority of the patients belonged to Caucasian population. For the study groups, comparative analysis was done and the confidence interval was determined (Table 1). A brief description of the studied SNP growth factors genes is presented in the table 2.

The study samples of chorionic tissue were obtained during medical abortion in women with a physiological course of pregnancy for a period of 5-9 weeks (26 samples) and with spontaneous

Table 1. Description of women groups

Criteria	Control		Miscarriage in the first trimester	
	145		143	
	Abs.	% (95% CI)	Abs.	% (95% CI)
Women with the first pregnancy	14	9.6 (4.8-14.5)	24	16.5 (10.5-22.6)
Women without delivery in anamnesis	30	20.7 (14.1-27.3)	50	34.5 (26.7-42.2)
Women with a pregnancy in anamnesis ended with a life birth	118	81.4 (75.0-87.7)	83	57.3 (49.9-65.3)
Women with a missed abortion in the first trimester in anamnesis	0	0	23	15.9 (9.9-21.8)

Table 2. Description of studied growth factor gene loci

Gene	SNP	Chromosome position	Gene localization	Expression
<i>VEGFA</i>	rs699947 (-2578C>A)	6:43768652	Promoter region	Decreases gene expression
	rs2010963 (-634G>C)	6:43770613	5'UTR	Increases gene expression
<i>TGFBI</i>	rs1800471 (915G>C)	19:41352971	Exon 1	Missense mutation (25Arg>Pro) increases gene expression

termination of pregnancy (35 samples) in the first trimester of pregnancy. Tissues were obtained in the operation room, isolated right after separation, immersed in liquid nitrogen and stored at a temperature of -80°C until the release of nucleic acids.

DNA was isolated using DNA-express kit (Lytech, Russia). The allelic variants $-634\text{G}>\text{C}$ (rs2010963) of the *VEGFA* and $915\text{G}>\text{C}$ (rs1800471) of the *TGFBI* were detected by allele-specific PCR using SNP-express reaction kits (Lytech, Russia). The assay is based on carrying out the amplification reaction with the two allele-specific primers. The PCR products were analyzed by horizontal 3% agarose gel electrophoresis. Gel images were captured using GelDoc XR system (Bio-Rad, USA). SNP- $2578\text{C}>\text{A}$ (rs699947) of the *VEGFA* was detected by the real-time PCR method using the "Synthol" reagents (Russia) according to the manufacturer's instructions. The data was analyzed on the CFX-96 (Bio-Rad, USA).

Total RNA was extracted by the acid guanidinium thiocyanate phenol method (Chomczynski and Sacchi, 1987). Upon isolation, RNA was immediately treated with DNase I (Syntol, Russia). RNA integrity was assessed using non-denaturing 1.5% agarose gel electrophoresis. Clear 18S and 28S bands were observed with no signs of RNA degradation. The RNA was reverse transcribed immediately following the RNA isolation and the DNase treatment using the "RT kit" (Syntol, Russia) with the template denaturation step and the oligo (dT) primer. Reverse transcription (With M-MLV enzyme) was performed during 50 min incubation at 42°C for 50 min, followed by duration of 92°C for 10 min. cDNA samples were stored at -20°C .

Primers and probe designing for analysis of gene expression was carried out using Primer 3 program (<http://frodo.wi.mit.edu/primer3/>). Primers were prepared by Syntol (Russia). The forward and reverse primers and probes used sequence are presented in table 3. *VEGFA* and *TGFBI* genes

expression levels were determined by real-time PCR on CFX96 (Bio-Rad, USA).

Statistical analysis: Hardy-Weinberg equilibrium analyses were performed using Hardy-Weinberg equilibrium calculator in www.oege.org/software/Hardy-Weinberg (22). Differences in distribution of allele variants between studied groups were assessed by χ^2 -analyses. The $p \leq 0.05$ was considered statistically significant. To evaluate pregnancy loss risk, odd ratios (OR) were calculated. OR was indicated with 95% confidence interval (CI) (23). Persons having more than one risk allele or genotype, may have a higher risk of pregnancy loss; therefore, gene-gene interactions were investigated. Gene-gene interactions were analyzed using the multifactor dimensionality reduction (MDR) method (24). MDR is well suited for genetic studies of multifactorial and polygenic diseases using relatively small ranges of patients and healthy ones. It allows to determine the nature of intergenic interactions. The method is used to model high-order genomic interactions that could not be estimated using traditional (Parametric) methods.

Statistical analysis of data of gene expression was performed by $2^{-\Delta\Delta\text{Ct}}$ method by Livak and Schmittgen (25). It shows the multiplicity of changes in genes expression level in the compared

Table 3. Sequence of PCR probes and primers

Gene	Sequence of PCR probes and primers
<i>VEGFA</i>	Forward 5' - GGATGTCTACCAGCGCAGC -3'
	Reverse 5' - TCTGGGTACTCCTGGAAGATGTC -3'
	ProbeFam- TCTGCCGTCCCATTGAGACCCTG-RTQ-1
<i>TGFBI</i>	Forward 5' - ATGGCATGAACCGGCCTT -3'
	Reverse 5' - AGGTCCTTGCGGAAGTCAA -3'
	ProbeFam- CGCCGAGCCCTGGACACCA -RTQ-1
<i>GAPDH</i>	Forward 5' -AGGTCGGAGTCAACGGATTT-3'
	Reverse 5' -ATCGCCCCACTTGATTTTGG-3'
	Probe Fam-GGCGCCTGGTCACCAGGGCT-BHQ1

Table 4. The frequency of alleles and genotypes (abs., %) for polymorphic variant -2578C>A of VEGFA gene in the blood cells of women with miscarriage

Genotype	Control	Miscarriage	χ^2 (P) *	OR (95% CI)
C/C	45 (31.0)	23 (18.7)		0.51 (0.29–0.91)
C/A	62 (42.8)	68 (55.3)	6.14 (0.05)	1.66 (1.02–2.69)
A/A	38 (26.2)	32 (26.0)		0.99 (0.57–1.71)
-2578A allele	0.476	0.537	1.96 (0.16)	1.28 (0.91–1.79)
HWE, χ^2 (P)	2.96 (0.09)	1.53 (0.22)		

* χ^2 : Comparison of frequencies of genotypes and alleles with the control

samples. All gene expression values (Δ Ct) in sample ranges were compared with each other as the results of the range pairs. For the confirmation of the statistically significant differences between sample ranges, the Mann-Whitney U-test was used.

Results

Genotypes and alleles frequencies for studied genes are shown in tables 4, 5 and 6. The distributions of all genotypes in each group were in Hardy-Weinberg equilibrium.

An association was found between the presence of polymorphism -2578C>A of the VEGFA gene in the genome with the miscarriage risk. The proportion of heterozygotes was 42.8% in the control and 55.3% in the comparison group for the -2578C>A polymorphism of VEGFA. Women with the CC genotype have a reduced risk of miscarriage in the first trimester. For women with the CA genotype, the risk of miscarriage was 1.66 (95% CI 1.02-2.69). The proportion of women with genotype AA was 26% in both groups. The -2578A allele frequency was 0.476 in the control group and 0.537 in the comparison group, respectively (Table 4).

The study of alleles and genotypes frequency distribution of VEGFA -634G>C and TGFBI 915C>G polymorphisms among women with pregnancy loss in the first trimester revealed no differences in comparison with control group (Table 5).

The results of the study of growth factor genes genotypes and alleles frequencies in chorion cells are presented in table 6. Genotypes and alleles frequencies distribution in chorion cells of all studied polymorphisms corresponded to the Hardy-Weinberg equilibrium. There were no significant differences in VEGFA and TGFBI genes polymorphisms genotypes and alleles frequencies between the studied groups.

Simultaneous presence of several candidate genes polymorphic variants, whose proteins par-

ticipate in common metabolic pathways, can lead to new phenotype formation. In this regard, multi-factor dimensionality reduction (MDR) method was used. Gene-gene interaction model was considered valid if its Cross Validation Consistency was not less than 9/10. Two models of allelic variants interaction were revealed with a change in the risk of miscarriage in the first trimester: VEGFA (-634G>C), VEGFA (-2578C>A) and VEGFA (-634G>C), VEGFA (-2578C>A), TGFBI (936C>T) (Cross Validation Consistency 10/10, p=0.0001).

Both two- and three-locus models of interaction of genes VEGFA and TGFBI identify the risk genotypes for pregnancy loss. Accordingly, it was shown that the VEGFA -2578C allele homozygote was associated with a reduction of miscarriage risk in the first trimester (OR 0.51, 95% CI 0.29–0.91) (Table 4). The miscarriage risk decreased with simultaneous presence of the VEGFA -634CG and VEGFA -2578CC genotype (OR 0.38, 95% CI 0.19–0.78, p=0.01) (Figure 1) and VEGFA -2578CC, VEGFA -634CG, TGFBI 936CC genotype (OR 0.45, 95% CI 0.21–0.96, p=0.057) (Figure 2).

The studied SNPs alter VEGFA and TGFBI genes expression (16, 26, 27). In this regard, the changes in the mRNA level of these genes were investigated in chorion and decidua of women with pregnancy loss in the first trimester compared with normal pregnancy. The mRNA level of VEGFA in decidual tissue does not differ from that in chorionic tissue in physiological pregnancy (p=0.61). If the early stages of embryonic development is disturbed, the intensity of expression of the VEGFA gene in tissues of maternal and em-

Table 5. The frequency of alleles and genotypes (abs., %) for polymorphic variants of VEGFA and TGFBI genes in the blood cells of women with miscarriage

VEGFA -634G>C				
Genotype	Control	Miscarriage	χ^2 (P) *	
G/G	70 (48.3)	77 (53.8)		
G/C	68 (46.9)	51 (35.7)	5.66 (0.06)	
C/C	7 (4.8)	15 (10.5)		
-634C allele	0.283	0.283	0 (0.99)	
HWE, χ^2 (P)	3.54 (0.06)	2.11 (0.15)		
TGFBI 915C>G				
C/C	121 (83.4)	110 (81.5)		
C/G	23 (15.9)	23 (17.0)	0.50 (0.78)	
G/G	1 (0.7)	2 (1.5)		
915G allele	0.086	0.100	0.32 (0.57)	
HWE, χ^2 (P)	0.01 (0.93)	0.39 (0.53)		

* χ^2 : Comparison of frequencies of genotypes and alleles with the control

Table 6. The frequency of alleles and genotypes (abs., %) for polymorphic variants of *VEGFA* and *TGFBI* genes in chorionic cells

<i>VEGFA</i> -634G>C			
Genotype	Control	Miscarriage	χ^2 (P) [*]
G/G	13 (50)	20 (57.1)	1.52 (0.47)
G/C	12 (46.2)	15 (42.9)	
C/C	1 (3.8)	0 (0)	
-634C allele	0.269	0.214	0.5 (0.48)
HWE, χ^2 (P)	0.78 (0.38)	2.6 (0.11)	
<i>TGFBI</i> 915C>G			
C/C	21 (80.8)	21 (61.8)	2.53 (0.28)
C/G	5 (19.2)	13 (38.2)	
G/G	0 (0)	0 (0)	
915G allele	0.096	0.191	2.09 (0.15)
HWE, χ^2 (P)	0.29 (0.59)	1.90 (0.17)	

* χ^2 : Comparison of frequencies of genotypes and alleles of the control

bryonic origin is also the same ($p=0.56$) (Figure 3). No changes of *VEGFA* expression were found in miscarriage compared with a physiological pregnancy (Table 7).

mRNA level of *TGFBI* was significantly higher in decidual tissue compared to chorion in normally progressing pregnancy ($p=0.003$) (Figure 4A). The *TGFBI* gene mRNA level in decidua and chorion is the same in the condition of miscarriage (Figure 4B). There were no significant differences in the *TGFBI* gene mRNA in decidual or chorionic tissues in miscarriage, relative to physiological pregnancy (Table 7).

A correlation analysis showed that in physiological pregnancy, the *VEGFA* gene expression level is directly correlated with the activity of *TGFBI* mRNA synthesis in decidua ($R=0.60$; $p=0.038$) (Figure 5A). In pregnancy loss, the dependence of mRNA synthesis of these genes in decidual tissue is preserved, but it has an inverse relationship ($R=-0.76$; $p=0.028$) (Figure 5B).

Discussion

Proper modeling of vascular reactions is necessary for normal placentation, embryo blood supply, its growth and development. SNP can lead to genes' activity change and protein product modification. It can lead to the pathologic course of pregnancy and even its interruption under the influence of certain conditions.

Data on the association between *VEGFA* polymorphisms and miscarriages are contradictory. *VEGFA* gene -2578C>A polymorphism is located in the promoter region, and the -2578C allele is

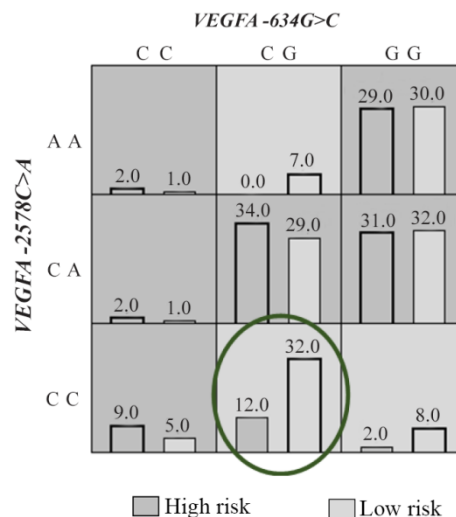


Figure 1. Distribution of high- and low-risk genotypes in the best two-locus model *VEGFA*(-634G>C (rs2010963), -2578C>A) (rs699947). High- (Dark shading) and low-risk (Light shading). The number of pregnancy loss subjects (Left black bar in boxes) and control subjects (Right black bar in boxes) is shown for each genotype combination. Significant low risk genotype is marked by the oval ($p<0.05$)

associated with a decreased level of gene expression (28). In heterozygotes, the level of *VEGFA* protein is slightly reduced and may not be enough for complete angiogenesis. This can lead to insufficient blood supply to the fetoplacental complex. Here, an association was found between the -2578C>A *VEGFA* polymorphism and pregnancy loss in the first trimester. Eller et al. identified a reduction in the -2578A allele frequency among women with recurrent pregnancy loss compared to fertile controls ($p=0.049$) (17). However, a number of researchers have found no significant correlations of this polymorphism with idiopathic recurrent miscarriage (18, 21, 29, 30). -634G>C polymorphism is located in the 5'-noncoding region. The -634C allele is associated with an increased level of *VEGFA* gene expression. It is supposed that any change in the *VEGFA* gene activity may lead to imbalance in the fetoplacental complex. There are data about the association of -634G>C polymorphism with unexplained recurrent pregnancy loss (17, 21). Other studies have shown a lack of -634G>C polymorphism association and miscarriage (18, 29). Our work showed a lack of association of -634G>C polymorphism and pregnancy loss too. The data are also contradictory for other functional polymorphisms of the *VEGFA* such as -1154G>A, 936C>T (18, 21, 29-31). Contradictions can be associated with the ethnic component

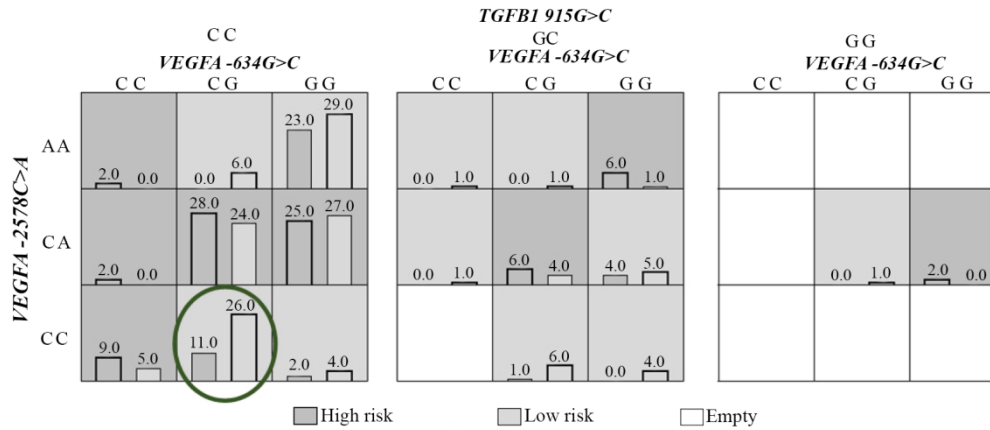


Figure 2. Distribution of high- and low-risk genotypes in the best three-locus model *VEGFA* (-634G>C (rs2010963), -2578C>A) (rs699947). High- (Dark shading) and low-risk (Light shading). The number of pregnancy loss subjects (Left black bar in boxes) and control subjects (Right black bar in boxes) is shown for each genotype combination. Significant low risk genotype is marked by the oval ($p < 0.05$)

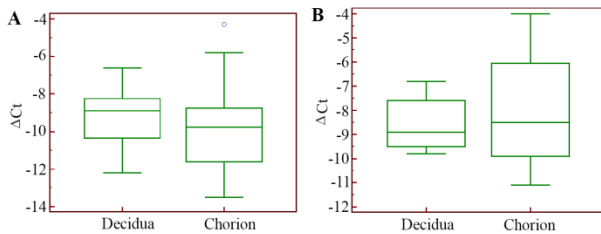


Figure 3. *VEGFA* gene expression level in the cells of chorionic and decidua tissues regarding GAPDH gene expression in normally progressing pregnancy (A) and pregnancy loss (B)

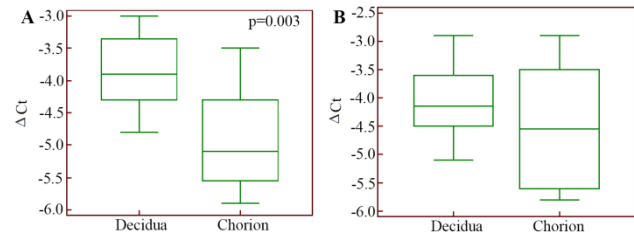


Figure 4. *TGFBI* gene expression level in the cells of chorionic and decidua tissues regarding GAPDH gene expression in normally progressing pregnancy (A) and pregnancy loss (B)

of miscarriage. Sun et al. have found a significant increased risk between *-1154G>A* (rs1570360) polymorphism and recurrent pregnancy loss in Caucasians. However, they found that *936C>T* (rs3025039) polymorphism was significantly associated with recurrent pregnancy loss in East Asians (17).

TGFBI is considered as one of the main regulators for monitoring regulatory T-cells that play a crucial role in maintaining physiological immune responses and, in addition, provide maternal tolerance to paternal fetal antigens (32). Transforming growth factor B regulates cell proliferation, differentiation, apoptosis, and homeostasis (33). Therefore, its significant role in the processes of maintaining pregnancy can not be excluded. Data for polymorphism *915G>C* *TGFBI* gene are few and contradictory. Magdoud et al. showed that polymorphism of *TGFBI 915G>C* increases the risk of recurrent miscarriage in Tunisian women (20). No association of this polymorphism with miscarriage was identified in Caucasians. Linsingen also failed to identify the associ-

ation of both *TGFBI* polymorphism *915G>C* and *869T>C* with miscarriage in women of Brazilian population (19). Amani has not found significant differences for women with spontaneous abortions of southern Iran for *Arg25Pro*, *Leu10Pro* and *Thr263Ile* polymorphisms (34).

Literature data on allele frequencies and genotypes investigated *VEGFA* and *TGFBI* SNPs in chorionic tissue in miscarriage in the first trimester are absent. The searching for the keywords "TGFB + chorion", "TGFB + placenta", "VEGFA + chorion", "VEGFA + placenta" and synonyms in the Scopus, Web of Science and PubMed databases did not yield any results. There are few studies about growth factors gene-gene interactions which are associated with a recurrent pregnancy loss in the first trimester.

SNP leads to growth factors genes' activity change, which affects their functioning. Disturbance of pregnancy course depends on the interaction of a growth factor genes complex products, and each of them contributes to the vascularization process. Therefore, it is important to esti-

Table 7. Rate of change of the expression level ($2^{-\Delta\Delta Ct}$) of the *VEGFA* and *TGFBI* genes in miscarriage, relative to physiological pregnancy

Gene	Decidual tissue	Chorionic tissue
<i>VEGFA</i>	1.6	3.2
<i>TGFBI</i>	0.85	1.2

mate gene-gene interactions. The MDR analysis data have shown the significance for two-locus and three-locus models of the *VEGFA* and *TGFBI* genes with miscarriage. Revealed two-locus and three-locus models of genotypes are associated with a reduced risk of miscarriage. Although a significant association only for *VEGFA* -2578C>A with pregnancy loss in the first trimester was indicated, three-locus model (*VEGFA* C-2578C, *VEGFAC*-634G, *TGFBI*C936C) may indicate the interaction of these SNPs in the implantation and placentation processes. The correlation analysis of *VEGFA* and *TGFBI* gene expression (Figure 5) also shows the complex involvement of these genes in pregnancy loss in the first trimester.

VEGF and *TGFBI* play an important role in the interphase pre-synthetic stage. *VEGF* stimulates cells growth and its division. *TGFBI* acts both as a stimulant and as a growth inhibitor (35-39). *VEGF* transmits signals through the phosphatidylinositol-3-kinase pathway (40, 41), mediated by interaction of PI3-K, PDK, Akt kinases (35, 36). This leads to increasing cyclin D expression and cell passing through the restriction site (35, 42). This promotes new endothelial cells formation and trophoblast invasion.

TGFBI induces the vascular endothelial growth factor expression (34, 43). Since the products of genes are interrelated in the metabolic pathways, a change in their functioning can trigger apoptosis, disturbances in the formation of blood vessels, and, as a consequence, impairment of syncytiotrophoblast functioning.

VEGF and *TGFBI* protein levels with lower *mRNA* levels were detected in the chorionic villi of spontaneously aborted samples compared with samples obtained by induced abortion (44, 45). It is possible that the risk of 915G>C *TGFBI*, *VEGFA*936 C>T and -2578C>A polymorphisms altered gene expression in the embryo. Consequently, this may lead to abnormal angiogenesis and spontaneous abortion. No association was found between pregnancy loss and growth factor genes polymorphisms frequencies for chorion cells. In the literature, similar studies were found

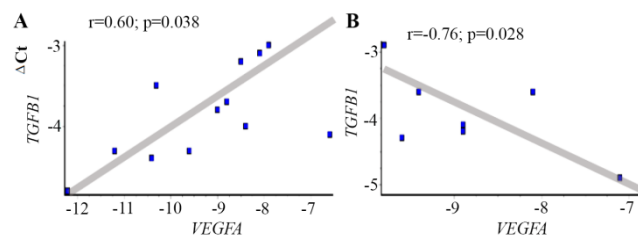


Figure 5. The ratio of the *VEGFA* and *TGFBI* genes expression level (ΔCt) in the cells of chorionic and decidual tissues regarding *GAPDH* gene expression in normally progressing pregnancy (A) and pregnancy loss (B)

as well. The frequencies for *VEGFA*936C>T and -2578C>A polymorphisms between fetus from miscarriage and control group were same (46).

VEGFA gene expression level is directly correlated with the activity of *TGFBI* mRNA synthesis in decidua in control. In pregnancy loss, the dependence of mRNA synthesis of these genes in decidual tissue has inverse relationship.

Down regulation of trophoblast cells differentiation through *TGFBI* signaling was shown by Morrish et al. (47). *TGFBI* inhibits the cyclin *D-Cdk4/6* and cyclin *E/Cdk2* complexes activity (48), which prevents the trophoblast cells invasion. *TGFBI* acts onto the cell cycle by two ways. On the one hand, it supports proliferation, and on the other, it triggers cell death. One of the mechanisms of this paradox is the relationship between *TGFBI* and *VEGF* mediated by *FGF-2*. The first way is *TGFBI* → *FGF-2* → *VEGF* → cell survival and proliferation (41). The second way is *TGFBI* → *VEGF* → *p38MAPK* → apoptosis (41, 49). Apoptosis activation is realized through a protein cascade. Yoo et al. have found that *GADD45b* participates in *TGFBI*-induced apoptosis by acting upstream of *p38* activation (49). Cao et al. have found that *TGFBI* induces *VEGF* gene expression through *Smad3* transcription factor (50). A change in the ratio of the concentrations of growth factors can disrupt the processes of cell division, apoptosis, and angiogenesis processes. Research data on the growth factors polymorphisms role indicates their significant contribution to pregnancy loss. It is clear that further research in the field is still warranted.

Conclusion

There were no changes of *VEGFA* expression in miscarriage compared with a physiological pregnancy. There were no significant differences in the *TGFBI* gene mRNA in decidual or chorionic tissues in miscarriage, relative to physiological

pregnancy. But the correlation analysis showed that in physiological pregnancy, the VEGFA gene expression level is directly correlated with the activity of TGFB1 mRNA synthesis in decidua. In pregnancy loss, the dependence of mRNA synthesis of VEGFA and TGFB1 genes in decidual tissue is preserved, but it has an inverse relationship.

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Conflict of Interest

None.

References

- Graham CH, Lysiak JJ, McCrae KR, Lala PK. Localization of transforming growth factor-beta at the human fetal-maternal interface: role in trophoblast growth and differentiation. *Biol Reprod* 1992;46(4): 561-72.
- Singh M, Orazulike NC, Ashmore J, Konje JC. Changes in maternal serum transforming growth factor beta-1 during pregnancy: a cross-sectional study. *Biomed Res Int*. 2013;2013:318464.
- Wen Z, Shen Y, Berry G, Shahram F, Li Y, Watanabe R, et al. The microvascular niche instructs T cells in large vessel vasculitis via the VEGF-Jagged1-Notch pathway. *Sci Transl Med*. 2017;9(399). pii: eaal3322.
- Wang Y, Liu Y, Fan Z, Liu D, Wang F, Zhou Y. IGFBP2 enhances adipogenic differentiation potentials of mesenchymal stem cells from Wharton's jelly of the umbilical cord via JNK and Akt signaling pathways. *PLoS One*. 2017;12(8):e0184182.
- Mattei MG, Borg JP, Rosnet O, Marme D, Birnbaum D. Assignment of vascular endothelial growth factor (VEGF) and placenta growth factor (PlGF) genes to human chromosome 6p12-p21 and 14q24-q31 regions, respectively. *Genomics*. 1996;32(1): 168-9.
- Sugimoto H, Hamano Y, Charytan D, Cosgrove D, Kieran M, Sudhakar A, et al. Neutralization of circulating vascular endothelial growth factor (VEGF) by anti-VEGF antibodies and soluble VEGF receptor 1 (sFlt-1) induced proteinuria. *J Biol Chem*. 2003; 278(15):12605-8.
- Paleolog EM. Angiogenesis in rheumatoid arthritis. *Arthritis Res Ther*. 2002;4(3):81-90.
- Yoo J, Ghiassi M, Jirmanova L, Balliet AG, Hoffman B, Fornace AJ Jr, et al. Transforming growth factor-beta-induced apoptosis is mediated by Smad-dependent expression of GADD45b through p38 activation. *J Biol Chem*. 2003;278(44):43001-7.
- Blobe GC, Schiemann WP, Lodish HF. Role of transforming growth factor beta in human disease. *N Engl J Med*. 2000;342(18):1350-8.
- Annes JP, Munger JS, Rifkin DB. Making sense of latent TGFbeta activation. *J Cell Sci*. 2003;116(pt 2):217-24.
- Euler-Taimor G, Heger J. The complex pattern of SMAD signaling in the cardiovascular system. *Cardiovasc Res*. 2006;69(1):15-25.
- Chow JFC, Lee KF, Chan ST, Yeung WS. Quantification of transforming growth factor beta1 (TGF beta1) mRNA expression in mouse preimplantation embryos and determination of TGFbeta receptor (type I and type II) expression in mouse embryos and reproductive tract. *Mol Hum Reprod*. 2001;7(11): 1047-56.
- Simpson H, Robson SC, Bulmer JN, Barbar A, Lyall F. Transforming growth factor beta expression in human placenta and placental bed during early pregnancy. *Placenta*. 2002;23(1):44-58.
- Agarwal I, Karumanchi SA. Preeclampsia and the anti-angiogenic state. *Pregnancy Hypertens*. 2011; 1(1):17-21.
- Schumacher A, Brachwitz N, Sohr S, Engeland K, Langwisch S, Dolaptchieva M, et al. Human chorionic gonadotropin attracts regulatory T cells into the fetal-maternal interface during early human pregnancy. *J Immunol*. 2009;182(9):5488-97.
- Kim SY, Lim JH, Park SY, Yang JH, Kim MY, Kim MH, et al. Transforming growth factor-beta 1 gene polymorphisms in Korean patients with preeclampsia. *Am J Reprod Immunol*. 2010;63(4): 291-8.
- Eller AG, Branch DW, Nelson L, Porter TF, Silver RM. Vascular endothelial growth factor-A gene polymorphisms in women with recurrent pregnancy loss. *J Reprod Immunol*. 2011;88(1):48-52.
- Sun Y, Chen M, Mao B, Cheng X, Zhang X, Xu C. Association between vascular endothelial growth factor polymorphism and recurrent pregnancy loss: a systematic review and meta-analysis. *Eur J Obstet Gynecol Reprod Biol*. 2017;211:169-76.
- Von Linsingen R, Bompeix EP, Bicalho Mda M. A case-control study in IL6 and TGFB1 gene polymorphisms and recurrent spontaneous abortion in southern Brazilian patients. *Am J Reprod Immunol*. 2005;53(2):94-9.
- Magdoud K, Granados V, Herbepin S, Messaoudi S, Hizem N, Bouafia WY, et al. Genetic variation in TGFB1 gene and risk of idiopathic recurrent pregnancy loss. *Mol Hum Reprod*. 2013;19(7):438-43.

21. Xu X, Du C, Li H, Du J, Yan X, Peng L, et al. Association of VEGF genetic polymorphisms with recurrent spontaneous abortion risk: a systematic review and meta-analysis. *PLoS One*. 2015;10(4):e0123696.
22. Rodriguez S, Gaunt TR, Day IN. Hardy-Weinberg equilibrium testing of biological ascertainment for mendelian randomization studies. *Am J Epidemiol*. 2009;169(4):505-14.
23. Petrie A, Bulman JS, Osborn JF. Further statistics in dentistry Part 8: systematic reviews and meta-analyses. *Br Dent J*. 2003;194:73-8.
24. Motsinger AA, Lee S, Mellick G, Ritchie MD. GPNN: power studies and applications of a neural network method for detecting gene-gene interactions in studies of human disease. *BMC Bioinformatics*. 2006;7:39.
25. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2^{-Delta}Delta C(T) method. *Methods*. 2001;25(4):402-8.
26. Watson CJ, Webb NJ, Bottomley MJ, Brenchley PE. Identification of polymorphisms within the vascular endothelial growth factor (VEGF) gene: correlation with variation in VEGF protein production. *Cytokine*. 2000;12(8):1232-5.
27. Sa-Nguanraksa D, Chuangsuwanich T, Pongpruttiapan T, Kummalue T, Rojananin S, Ratanawichitrasin A, et al. Vascular endothelial growth factor -634G/C polymorphism is associated with increased breast cancer risk and aggressiveness. *Mol Med Rep*. 2013;8(4):1242-50.
28. Shahbazi M, Fryer AA, Pravica V, Brogan IJ, Ramsay HM, Hutchinson IV, et al. Vascular endothelial growth factor gene polymorphisms are associated with acute renal allograft rejection. *J Am Soc Nephrol*. 2002;13(1):260-4.
29. Papazoglou D, Galazios G, Papatheodorou K, Liberis V, Papanas N, Maltezos E, et al. Vascular endothelial growth factor gene polymorphisms and idiopathic recurrent pregnancy loss. *Fertil Steril*. 2005;83(4):959-63.
30. Lee HH, Hong SH, Shin SJ, Ko JJ, Oh D, Kim NK. Association study of vascular endothelial growth factor polymorphisms with the risk of recurrent spontaneous abortion. *Fertil Steril*. 2010;93(4):1244-7.
31. Eller A, Branch DW, Nelson L, Silver R. The -634GC polymorphism in the regulatory 5' untranslated region (5' UTR) of the vascular endothelial growth factor (VEGF) gene is associated with unexplained recurrent pregnancy loss (RPL). *Am J Obstet Gynecol*. 2008;199(6):S81.
32. Li X, Shen L, Tan H. Polymorphisms and plasma level of transforming growth factor-Beta 1 and risk for preeclampsia: a systematic review. *PLoS One*. 2014;9(5):e97230.
33. Amani D, Dehaghani AS, Zolghadri J, Ravangard F, Niikawa N, Yoshiura K, et al. Lack of association between the TGF-beta1 gene polymorphisms and recurrent spontaneous abortion. *J Reprod Immunol*. 2005;68(1-2):91-103.
34. Marchenko ZhS, Lukina GV. Vascular endothelial growth factor role in the rheumatoid arthritis pathogenesis. *Sci Pract Rheumatol*. 2005;1:57-60.
35. Zhang H, Yee D. Insulin-like growth factor binding protein-1 (IGFBP-1) inhibits breast cancer cell motility. *Cancer Res*. 2002;62(15):4369-75.
36. Qiang YW, Yao L, Tosato G, Rudikoff S. Insulin-like growth factor I induces migration and invasion of human multiple myeloma cells. *Blood*. 2004;103(1):301-8.
37. Gwinn DM, Shackelford DB, Egan DF, Mihaylova MM, Mery A, Vasquez DS, et al. AMPK phosphorylation of raptor mediates a metabolic checkpoint. *Mol Cell*. 2008;30(2):214-26.
38. Appert-Collin A, Hubert P, Crémel G, Bennisroune A. Role of ErbB receptors in cancer cell migration and invasion. *Front Pharmacol*. 2015;6:283-92.
39. Ferrari G, Cook BD, Terushkin V, Pintucci G, Mignatti P. Transforming growth factor-beta 1 (tgf-beta1) induces angiogenesis through vascular endothelial growth factor (VEGF)-mediated apoptosis. *J Cell Physiol*. 2009;219(2):449-58.
40. Kyriakis JM. Making the connection: coupling of stress-activated ERK/MAPK (extracellular-signal-regulated kinase/mitogen-activated protein kinase) core signaling modules to extracellular stimuli and biological responses. *Biochem Soc Symp*. 1998;64:29-48.
41. Ferrari G, Pintucci G, Seghezzi G, Hyman K, Galloway AC, Mignatti P. VEGF, a prosurvival factor, acts in concert with TGF-beta1 to induce endothelial cell apoptosis. *Proc Natl Acad Sci USA*. 2006;103(46):17260-5.
42. West MJ, Stoneley M, Willis AE. Translational induction of the c-myc oncogene via activation of the FRAP/TOR signaling pathway. *Oncogene*. 1998;17(6):769-80.
43. Poniatowski ŁA, Wojdasiewicz P, Gasik R, Szukiewicz D. Transforming growth factor Beta family: insight into the role of growth factors in regulation of fracture healing biology and potential clinical applications. *Mediators Inflamm*. 2015;2015:137823.

44. Choi HK, Choi BC, Lee SH, Kim JW, Cha KY, Baek KH. Expression of angiogenesis- and apoptosis-related genes in chorionic villi derived from recurrent pregnancy loss patients. *Mol Reprod Dev.* 2003;66(1):24-31.
45. Pang L, Wei Z, Li O, Huang R, Qin J, Chen H, et al. An increase in vascular endothelial growth factor (VEGF) and VEGF soluble receptor-1 (sFlt-1) are associated with early recurrent spontaneous abortion. *PLoS One.* 2013;8(9):e75759.
46. Yalcintepe SA, Silan F, Hacivelioglu SO, Uludag A, Cosar E, Ozdemir O. Fetal Vegf genotype is more important for abortion risk than mother genotype. *Int J Mol Cell Med.* 2014;3(2):88-94.
47. Morrish DW, Bhardwaj D, Paras MT. Transforming growth factor beta 1 inhibits placental differentiation and human chorionic gonadotropin and human placental lactogen secretion. *Endocrinology.* 1991;129(1):22-6.
48. Hocevar BA, Brown TL, Howe PH. TGF- β induces fibronectin synthesis through a c-Jun N-terminal kinase-dependent Smad4-independent pathway. *EMBO J.* 1999;18(5):1345-56.
49. Yoo J, Ghiassi M, Jirmanova L, Balliet AG, Hoffman B, Fornace AJ, ET AL. Transforming growth factor-beta-induced apoptosis is mediated by Smad-dependent expression of GADD45b through p38 activation. *J Biol Chem.* 2003;278(44):43001-7.
50. Cao Y, Townsend CM, Ko T. Transforming growth factor-beta (TGF-beta) induces vascular endothelial growth factor (VEGF) and plasminogen activator inhibitor-1 (PAI-1) gene expression through Smad3 transcription factor. *J Am Coll Surg.* 2005; 201(3):S17-S18.