MMP20 Expression in Chorionic Tissue and Decidua of Women with Early Pregnancy Loss

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Citation

Abstract: MMPs play an important role in human reproduction, because in the early stages of embryogenesis the degradation and remodeling of extracellular matrix are the key processes. Only known function for MMP20 is dental enamel formation. But the data about its role in spontaneous abortion is very poor. Thus, the aim of this study was to investigate the association of Val275Ala (rs1784423) and A320C (rs2245803) polymorphisms MMP20 gene with pregnancy loss in the first trimester, and MMP20 gene expression analysis in decidual and chorionic tissues. A total of 132 women with spontaneous abortion in the first trimester (n=12) and progressing pregnancies in 5-9 week of gestation were taken after surgical termination of normal pregnancy. Our investigation have not detected association of gene polymorphisms MMP20 Val275Ala (rs1784423) and A320C (rs2245803) with miscarriage in the first trimester. Compared with chorionic tissue, the MMP20 expression was increased in decidua in both groups (p = 0.0037, P = 0.014 respectively). The results demonstrated the tissue-specific MMP20 expression in normal pregnancy and spontaneous abortion.

Key Words: MMP20, Gene expression, Spontaneous abortion, Chorionic tissue, Decidua
destroys amelogenin, the main protein component of tooth enamel matrix and thus plays an important role in the dental enamel formation and oral carcinogenesis.\(^1\)\(^2\)\(^3\)

The aim of this study was to investigate the association of **Val275Ala** (rs1784423) and **A320C** (rs2245803) polymorphisms of **MMP20** gene with pregnancy loss in the first trimester in women, and gene expression analysis in decidua and chorionic tissues.

**Materials and Methods**

Prior to inclusion in the study, all subjects underwent a standard diagnostic work-up. Women with the uterine abnormalities and polycystic ovary syndrome, previously diagnosed hypertension, diabetes, thyroid disease and autoimmune disorders, and infectious diseases during pregnancy were excluded from studied population. Women contacting with exogenous risk factors, such as alcohol, electromagnetic radiation, industrial noise, vibration, chemical pollutants were also excluded. All smokers also were excluded from studied population because there are data, that tobacco smoking is a factor that increases the risk of spontaneous abortion.\(^18\) After approval by institutional review board, 132 women with spontaneous abortion in the first trimester and 144 women with normally progressing pregnancies and without any history of spontaneous or missed abortion were studied. The participants willingly signed the informed consent.

Samples of chorionic and decidual tissues were taken after surgical termination by curettage of normally progressing pregnancies in 5-9 week of gestation (n=12) and spontaneous abortion in 5-9 week of gestation (n=12). Villous samples from the control group were obtained from women undergoing elective abortion for social reasons. Samples were stored at -80°C in aliquots for RNA isolation, and thawed only once to avoid degradation.

Genomic DNA was isolated using phenol chloroform method from decidual and chorionic tissue. Polymorphism **Val275Ala** (rs1784423) and **A320C** (rs2245803) **MMP20** gene were detected by allele-specific polymerase chain reaction method using SNP-express reaction kits (Lytech, Russia). The assay is based on carrying out the amplification reaction in pairs 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). Densitometry was performed using ImageJ (NIH, USA). The background was subtracted with the rolling ball radius of 50 pixels.

Total RNA from samples of decidua and chorionic tissue was extracted by the acid guanidium thiocyanate phenol method.\(^19\) After isolation, RNA was immediately treated with DNAsel (Roche, Switzerland). The concentration of the purified total RNA samples was determined using spectrophotometer SmartSpec Plus (BioRad, USA) at 260 nm.

The RNA was reverse transcribed immediately after the RNA isolation and the DNAsel treatment using the “RT” kit (Syntol, Russia) with the template denaturation step and the oligo (dT) primer. To carry out reverse transcription polymerase chain reaction reagents produced by Syntol (Russia) was used. The reaction mixture consisted of: 1 µl primer Random-6 (Syntol, Russia), 2 µg the total RNA, 10 µl H₂O, deionized, free of nucleases. For thermal denaturation step - 70 °C for 5 minutes. After primer annealing samples cooled on ice and the remaining reagents were added for reverse transcription, including: 10 µl 2.5 X reaction mixture, 1 µl reverse transcriptase MMLV-RT (50 U/µl); 1 µl RNase inhibitor (5 U/µl).

Probe without a reverse transcriptase served as negative control. Reverse transcription was performed for 50 min incubation at 55°C for 5 minutes, followed by duration of 92°C for 10 min. cDNA samples were stored at -20°C. The inactivation of the reverse transcriptase MMLV-RT was performed at 95°C for 8 minutes. The obtained cDNA was used for amplification.

**MMP20** gene expression level was determined by real-time PCR on CFX96 (Bio-Rad, USA). Primers was purchased from Syntol (Russia). The forward and reverse primers and probes used sequence are presented in the Table 1.

The amplification reaction was conducted in two replicates for each sample. Cycling parameters were the following: 94°C for 10 min; 40 cycles: 60°C for 50 c, 94°C for 15 c. The reaction mixture (Syntol (Russia)) consisted of: 2.5 µl dNTPs (2.5 mM), 2.5 µl 10x PCR buffer, 2.5 µl MgCl₂ (25 mM), 1.0 µl primer (10 pmol/µl), 0.5 µl probe with a fluorescent label (10 pmol/µl), 0.5 µl Tag DNA-polymerase (5 U/µl), deionized H₂O to 22.0 µl and 3.0 µl cDNA sample.

### Table 1: Sequence of PCR probes and primers

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sequence of PCR probes and primers</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MMP20</strong></td>
<td>Forward 5’- AAGTCGACCGGGAAGTTAGA -3’</td>
</tr>
<tr>
<td></td>
<td>Reverse 5’- CAGGGAGAGGCGATAATTG -3’</td>
</tr>
<tr>
<td></td>
<td>Probe Fam-TCGCTGTGAGGTCTCAGTGATGGC-TAMRA</td>
</tr>
<tr>
<td><strong>GAPDH</strong></td>
<td>Forward 5’- AGGTCCGAGTCAACCGGATT -3’</td>
</tr>
<tr>
<td></td>
<td>Reverse 5’- ATGCCTCCCATCTGATGGTGGTGTGG -3’</td>
</tr>
<tr>
<td></td>
<td>Probe Fam-GGCCGCTTGGTCAAGGGCT-BHQ1</td>
</tr>
</tbody>
</table>

Hardy-Weinberg equilibrium analyses were performed using Hardy-Weinberg equilibrium calculator in www.oege.org/software/Hardy-Weinberg.\(^20\) Differences in allele variants between studied groups were assessed by X²-analyses. P-value <0.05 was considered statistically significant. To evaluate pregnancy loss risk we calculated odd ratios (OR). OR was indicated with 95% confidence interval (CI).

Statistical analysis of gene expression data in tissues was performed by X²-ΔΔCt method by Livak K and Schmittgen T.\(^21\)

**Results**

Frequencies of genotypes and alleles for polymorphisms **Val275Ala** (rs1784423) and the **A320C** (rs2245803) of the gene **MMP20** are shown in Table 2.

The distributions of all genotypes in each group were in Hardy-Weinberg equilibrium. Heterozygotes dominated in women with pregnancy loss with **Val275Ala** among women with miscarriage was 50%, the same value in the control group. The nature of the frequency distribution of genotypes and alleles for the polymorphism **Val275Ala** **MMP20** gene in the comparison group equal to the control group.

Differences between the two groups of women for polymorphism **A320C** (rs2245803) of the gene **MMP20** was not detected.
Matrix metalloproteinases gene polymorphisms increase early pregnancy loss risk. Matrix metalloproteinases gene expression in chorionic and decidual tissues regarding GAPDH gene expression at normally progressing pregnancy (A) and pregnancy loss (B). Regeneration of MMP activity can be crucial for successful implantation and placentation. According to numerous studies using animal models, many MMP subtypes expressed not only in invading trophoblast cells, but also in the endometrial stromal cells and natural killer (NK) in the maternal uterus tissue. Anacker J et al have shown that all known human MMPs except MMP20 were expressed at the mRNA level in human decidua during pregnancy. The expression of MMP8 and MMP13 during pregnancy is shown. The expression of MMP8 and MMP13 during pregnancy is shown. MMP2 and MMP9 play special role in trophoblast behavior regulation. MMP1 and MMP3 are also detected in the extracellular trophoblast, which contribute to blastocyst implantation. MMP27 and MMP28 are expressed predominantly in trophoblast cells. Then it was shown that MMP25 and MMP20 are expressed in invading extravillous cytotrophoblast cells. Matrix metalloproteinases gene polymorphisms increase early pregnancy loss risk. (11) The frequency of Table 2: The frequency of alleles and genotypes (absolute value, %) for polymorphic variants of MMP20 gene in the blood cells of women with miscarriage

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Control</th>
<th>Miscarriage</th>
<th>OR (95% CI)</th>
<th>$X^2$ (p)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Val/Val</td>
<td>33 (22.9)</td>
<td>33 (25.0)</td>
<td>1.12 (0.64 – 1.95)</td>
<td>0.23 (0.89)</td>
</tr>
<tr>
<td>Val / Ala</td>
<td>77 (53.5)</td>
<td>67 (50.8)</td>
<td>0.90 (0.56 – 1.44)</td>
<td>0.43 (0.57)</td>
</tr>
<tr>
<td>Ala / Ala</td>
<td>34 (23.6)</td>
<td>32 (24.2)</td>
<td>1.04 (0.60 – 1.80)</td>
<td>0.99 (0.81)</td>
</tr>
<tr>
<td>275Ala allele</td>
<td>0.503</td>
<td>0.496</td>
<td>0.97 (0.70 – 1.36)</td>
<td>0.03 (0.86)</td>
</tr>
</tbody>
</table>

A320C (rs2245803)

<table>
<thead>
<tr>
<th>Gene</th>
<th>Control</th>
<th>Miscarriage</th>
<th>OR (95% CI)</th>
<th>$X^2$ (p)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>16 (11.2)</td>
<td>19 (14.8)</td>
<td>1.38 (0.68 – 2.82)</td>
<td>1.37 (0.5)</td>
</tr>
<tr>
<td>AC</td>
<td>59 (41.3)</td>
<td>56 (43.8)</td>
<td>1.11 (0.68 – 1.79)</td>
<td>0.83</td>
</tr>
<tr>
<td>CC</td>
<td>68 (47.6)</td>
<td>53 (41.4)</td>
<td>0.78 (0.48 – 1.26)</td>
<td>0.23</td>
</tr>
</tbody>
</table>

Table 3: Rate of change of the expression level ($2^{\Delta\Delta Ct}$) of gene MMP20 in decidual tissue relative to the chorionic tissue

<table>
<thead>
<tr>
<th>Gene</th>
<th>Normally progressing pregnancy</th>
<th>Miscarriage pregnancy loss</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMP20</td>
<td>123.6</td>
<td>54.6</td>
</tr>
</tbody>
</table>

Table 4: Rate of change of the expression level ($2^{\Delta\Delta Ct}$) of gene MMP20 in miscarriage, relative to physiological pregnancy

<table>
<thead>
<tr>
<th>Gene</th>
<th>Decidual tissue</th>
<th>Chorionic tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMP20</td>
<td>0.37</td>
<td>0.83</td>
</tr>
</tbody>
</table>

Discussion

Normally developing pregnancy is accompanied by alteration of tissues in the mother’s body. The embryo is characterized by more pronounced changes in structure. Involving not only cell division that associated with the formation of tissues and organs anlage, but also cell migration, extracellular trophoblast invasion into the maternal tissues and placenta. All these processes require tissue intercellular space and cell structure reorganization, which in turn is caused by proteolytic enzymes functioning, including metalloproteinases and their inhibitors. Changes in MMP genes expression on mRNA and functionally active protein levels lead to disruption of trophoblast invasion process. Many early pregnancy loss cases are accompanied by anatomical defects of placenta, such as thin and fragmented trophoblast, violation of cytotrophoblast invasion into the uterus and incomplete closure of the spiral arteries, leading to premature excessive blood supply to the developing placenta. Excessive blood filling in intervillous space causes a direct mechanical effect in the villous tissue, and oxygen-dependent damage in the trophoblast followed by apoptosis activation. (26, 27) As a result, disruption in syncytiotrophoblast functioning and placental abruption becomes possible. In addition, the invasive potential of decidual cells may explain the violation of a current pregnancy. Regulation of MMP activity can be crucial for successful implantation and placentation. According to numerous studies using animal models, many MMP subtypes expressed not only in invading trophoblast cells, but also in the endometrial stromal cells and natural killer (NK) in the maternal uterus tissue. Anacker J et al have shown that all known human MMPs except MMP20 were expressed at the mRNA level in human decidua during pregnancy. The expression of MMP8 and MMP13 during pregnancy is shown. MMP2 and MMP9 play special role in trophoblast behavior regulation. MMP1 and MMP3 are also detected in the extracellular trophoblast, which contribute to blastocyst implantation. MMP27 and MMP28 are expressed predominantly in trophoblast cells. Then it was shown that MMP25 and MMP20 are expressed in invading extravillous cytotrophoblast cells. Matrix metalloproteinases gene polymorphisms increase early pregnancy loss risk. (11) The frequency of
polymorphisms MMP2 -735 C/T and MMP9 -1562 C/C significantly increased in women with idiopathic recurrent pregnancy loss. (10,11) We have shown that the A-802G MMP9 polymorphism is associated with an increased risk of miscarriage in the first trimester. (30) Cohen M et al investigated the level of mRNA MMP gene and protein expression during pregnancy and suggested that decidual stromal cells have higher expression levels of MMP than trophoblast cells, and decidual tissue susceptibility to invasion is probably increased in the presence of cytotrophoblast cells. In contrast, decidual cells don’t impact the invasive properties of cytotrophoblasts. (28) Our investigation have not detected association of gene polymorphisms MMP20 Val275Ala (rs1784423) and A320C (rs2245803) with miscarriage in the first trimester. Also in our findings we have shown the tendency of reducing the MMP20 gene mRNA level in decidua tissue in spontaneous abortion, although the data are not statistically significant differences.

Conclusion
We have shown for the first time that MMP20 is expressed in embryonic tissues and decidua with significantly higher expression level in decidual tissue compared with chorionic. Our data revealed the tissue-specific MMP20 expression in normal pregnancy and spontaneous abortion. It points the role of MMP during pregnancy This highlights the relevance of further studies.

Acknowledgement
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Conflict of Interest: None.

References

