Study of polymorphic variants of the TNF gene in pregnant women with mycoplasma infection in the Kazakh population

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Abstract

A molecular genetic study of DNA in 98 pregnant women with mycoplasma infection and 100 healthy pregnant women was carried out. The results of the study revealed that the presence of the homozygous mutant genotype AA increases the risk of pro-inflammatory processes in the body by 6.7 times, and the carriage of GA genotypic variants increases the risk of its occurrence by 2.6 times.

Keywords

Mycoplasma infection, pregnancy, TNF

Introduction

The problem of infection is still important and relevant in social health, as well as in the scientific and practical aspects. In recent years, a dramatic increase of infections, including mycoplasma is observed throughout. Despite significant advances in their diagnosis and treatment, these infections remain very important in the structure of obstetric pathology and perinatal morbidity and mortality [1,2].

The review of local and foreign literature indicates inconsistent evidence of clinical significance of mycoplasma infection in obstetrics. Some authors tend to interpret mycoplasmosis as normocenosis; others believe that it is the cause of miscarriage, fetal death, malformations of the central nervous system of the fetus.

Currently, attention is being focused at the molecular and genetic aspects of the formation of long-term persistence of microorganisms. Persistent infections are characterized by widespread, complexity of pathogenesis, severity of complications and lack effective treatment. Therefore, the actual action would be the search of mechanisms, contributing to the development and maintenance of persistent infections in the body. There are several reports in the literature, showing the change of tumor necrosis factor (TNF) in viral infections, exacerbation of chronic infections. Search of susceptibility markers for infections among alleles of TNF is a new, little-studied aspect of the study. In this connection, molecular genetic research in cases of mycoplasma infection shall be efficient and resourceful.

Material and methods

DNA samples from 98 pregnant women with mycoplasma infection and 100 healthy pregnant women as the check sample were used as a material for molecular genetic studies to identify single nucleotide polymorphism 308G>A of the TNF-α gene. All of the research subjects belonged to the Kazakh nationality. Blood sampling was performed after a medical examination in a voluntary order.

Molecular genetic study was carried out in several stages. Isolation of DNA from peripheral blood of the subjects was carried out at the DNA/RNA 6100 station of the Applied Biosystems production, according to the protocol supplied with the reagents.

Diagnostic kit of primers and restriction enzymes, designed for the analysis of 100 DNA samples of LLC “Center for Molecular Genetics” (Moscow, Russia) (kit code – TNF-α) was used to analyze the polymorphism 308G>A of the TNF-α gene.

Amplification of DNA in polymerase chain reaction (PCR) with using specific oligonucleotide primers was performed on the “BioRad” amplifier (Hercules, CA) in 23 mcL reaction mixture. PCR was performed according to the scheme: initial denaturation (95°C, 5 min); 30 cycles of amplification with the following parameters: (1) denaturation – 94°C, 1 s; (2) annealing – 66°C, 1 s; (3) synthesis – 72°C, 1 s; followed by incubation at 72°C for 7 min.

Amplified fragments were subjected to enzymatic hydrolysis using restriction enzyme Sty I, which facilitates the precipitation in the presence of a restriction site in case of mutation. In 3 mcL of the restriction mixture was added 12 mcL of the amplifyct and the resulting mixture was subjected to centrifugation after thermostating at 37°C overnight. The resulting hydrolysates were evaluated by performing vertical electrophoresis in 7% polyacrylamide gel (PAA) with a voltage of 160 V/cm during 50–60 min. As electrophoresis buffer, a single tris-borate buffer (1xTBE) was used. Samples of the amplified DNA before restriction and the molecular weight marker 100 bp were used as the marker upon completion of electrophoresis, the gel was stained with ethidium bromide (0.1 mkg/ml) for 10–15 min, and visualized under transmitted UV light. Detection of the results of electrophoresis was performed using “System of Gel Documentation – GelDoc” (BioRad, Hercules, CA).
The length of the digested fragments is recorded on the gel in the form of strips of the following size: in the case of allele G – 128 + 20 bp and in the case of allele A – 148 bp. The length of the amplified fragment is 148 bp (base pair). After restriction analysis, gel strips are recorded:

- In the case of allele G – 128 and 20 bp;
- In the case of allele A – 148 bp.

In case, when the subject has unchanged normal GG genotype on the gel instead of the original 148-bp fragment, 128-bp fragment is recorded. In the presence of a heterozygous mutation, A/G original fragment at 148 bp is replaced by strips at 128 and 20 bp, in the presence of functionally impaired (mutant) allele A in the homozygous state (genotype AA) on the PAA gel is recorded 148 bp fragment.

Thus, as a result of molecular genetic analysis of the subject, one of the three genotypic variants of the gene TNF-α on polymorphism G308A is revealed.

Allele frequencies and its errors were determined using the statistical package of the BIOSYS-1 program [3,4].

Strength of the association was expressed as values of relative risk OR (relative risk), calculated as the odds ratio (OR) by the formula:

$$ OR = \frac{(a \times d)}{(b \times c)} $$

Where a – number of persons with the marker, b – with no genetic marker of inmate patients, and c and d – number of persons with the presence and absence of this marker in healthy patients, respectively; in the case when one of the indicators is 0, the relative risk is calculated by using a modified formula:

$$ OR = \frac{[(2a + 1)(2d + 1)]}{[(2b + 1)(2c + 1)]} $$

Where OR = 1 – equals no association, OR > 1 is considered as a positive association with the disease allele or genotype (“risk factors”) and OR < 1 as a negative association (“stability factor”), i.e. consider the value of OR > 1 as a risk factor for this disease and OR = 1 as a preventive factor.

Statistical processing included calculation of average values and their errors, dispersion, and correlation analysis. Reliability of differences was assessed using the Student’s t-test and χ² of Yates. Statisticheskaya processing includes calculating the mean values and their errors, dispersion, and correlation analysis. Reliability of differences was assessed using the Student t-test and χ² of Yates.

**Results and discussion**

The frequency of normal GG genotype in the study group was 12.8 ± 3.4% and is lower in comparison with the control group (30.0 ± 4.6%), the differences were statistically significant (χ² = 8.5; p = 0.004).

The frequency of heterozygous genotype AG in the study group was 52.1 ± 3.6%, which has no significant differences from those of the control group (64.0 ± 4.8%) (χ² = 0.85; p = 0.4).

Significantly higher frequencies of the homozgyous for the mutant allele genotype AA – 19.3 ± 3.9% were obtained, and the mutant allele A – 52.1 ± 3.6% in the study group in contrast to the data of the control group (6.0 ± 2.4, 38.0 ± 3.4, χ² = 7.8 and χ² = 5.9, respectively, p < 0.05).

The frequency of the normal allele G is 47.9 ± 3.6% in the study group and is significantly lower than in the control group (62.0 ± 3.4) (χ² = 7.8; p = 0.005).

Thus, the frequency of normal GG genotype and G in the study group was significantly lower, and the frequency of the mutant (pathological) AA genotype and A allele was significantly higher in patients in contrast to the healthy control group (p < 0.05).

Data on the distribution of genotypes of polymorphism 308G>A of TNF-α gene in the study and control group confirm the indicators of the relative risk OR, that allows to determine the risk of disease with the carriage of different genotypes and haplotypes.

In case of carriage of AA and GA genotypes, the frequency of which was significantly higher among pregnant women of the main group, it was revealed that in the presence of homozygous mutant genotype AA, the risk of pro-inflammatory processes in the body is increased by 6.7 times and carriage of genotypic variants of GA by 2.6 times.

The results indicate the presence of a pathological relationship between carriage of the mutant allele A of TNF-α gene with the development of the studied pathology. Carriage of unfavorable genotypes AA and GA of TNF-α gene is statistically significant genetic risk factor for the development of inflammatory processes in the body of a pregnant woman, as it was confirmed by the relative risk OR coefficients.

The resulting data speak that in pregnant women, depending on the carriage of the mutant allele in the homozygous and heterozygous state, before pregnancy it is possible to carry out the correction of immune changes for the prevention of mycoplasma infection, as other markers (IL-1, IL-6, etc.) appear later only during pregnancy.

**Conclusion**

Our research data show that in pregnant women with mycoplasma infection, long-term persistence of imbalances in developing the production of pro-inflammatory cytokines in peripheral blood and locally in the cervical mucus – which is compounded in carriage of the homozygous AA genotype heterozygous and GG genotype of TNF-α – on the level of systemic immunity, leads to an imbalance of all T-cell immunity in the mother–placenta–fetus system.

**Declaration of interest**

The authors have no relevant financial, personal, political, intellectual or religious interests.

**References**

1. Kira EF. Bacterial vaginosis. St. Petersburg: 2001. 359 p.
2. Prilepskaya VN, Fofanova IV. Mycoplasma infection and pregnancy. Obstet Gynecol 2007;4:S.5–8.
3. Swoford DL, Selanger RB. BIOSYS-1, a FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematic. J Heredity 1990;72:281–3.
4. Sergiyenko VI, Bondareva IB. Mathematical statistics in clinical studies. 2nd ed. Moscow: GEOTAR MEDITSINA; 2000. 138 p.