INFLUENCE OF MOLECULAR-GENETIC FACTORS ON IMMUNE INDICATORS IN MEN WITH IMPAIRED FERTILITY

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Genetic factors account for 30-50% of all cases of severe forms of male infertility. Spermatogenesis is a complex biological process that depends on a precisely controlled cascade of activation and deactivation of certain genes. The result of these genes work is the process of maturation of spermatozoa from progenitor cells (spermatogones). Among gene factors, associated with azoospermia, CFTR gene mutations / variants are the most common. They can cause cystic fibrosis (CF) and congenital bilateral aplasia of vas deferens (CBAVD) syndrome, leading to bilateral aplasia and obstruction of the vas deferens. CF is one of the most common monogenic diseases with an autosomal recessive mode of inheritance. More than 95% of men with CF and all patients with CBAVD have infertility due to obstructive azoospermia. In 88% of men with CF, bilateral obstruction of the vas deferens at the level of the epididymis and / or vas deferens and aplasia of the seminal vesicles are noted. Obstructive azoospermia, observed in men in 25% of cases, is a consequence of unilateral or bilateral congenital absence of the vas deferens, which arose due to mutations in CFTR gene.

The purpose of the research is to study the features of immune-genetic factors and their combinations that affect male reproductive function.

Materials and methods. 135 men, aged 28 to 45 years with impaired fertility were examined, who underwent standard spermological, molecular- genetic (CFTR gene polymorphisms) and immunological (IL-2, IL-6 and TNF-α levels) studies. According to the results of immune-genetic studies for determination of CFTR gene polymorphism, 2 groups were formed: the first group - 117 men in whom CFTR gene polymorphism was not detected and the second group - 18 men with CFTR gene mutations. Molecular genetic research was performed on DNA, extracted from peripheral blood lymphocytes, using a kit of reagents for extraction of DNA Prep100 according to the manufacturer's protocol. CFTR gene was analyzed for the most common eight mutations. Concentration of pro-inflammatory cytokines (IL-2, IL-6 and TNF-α) was carried out in the ejaculate by EIA method. The control group consisted of 20 apparently healthy men who were married and had children

Results and discussion. Analysis of clinical data showed that the proportion of patients with primary infertility was 79, 4%, with secondary – 19, 8%. Duration of infertility – 4,9 ± 3,7 years (min – 1 year, max – 10 years). When studying the age history, it was found that the average age of men was 32, 8 ± 6,1 years . The minimum age of men in the first group was within 22 years, and the maximum age was 35 years. For men in group two, the minimum age was 26 years, and the maximum - 42 years. Asthenozoospermia prevailed in the structure of pathozoospermia in men in both the first and second groups, followed by oligozoospermia, asthenoteratozoospermia, teratozoospermia and azoospermia.

In order to study the specifics of the genetic contribution of CFTR gene to the development of male infertility, this study analyzed eight the most frequent mutations of CFTR gene, associated with cystic fibrosis. Molecular genetic studies were carried out in 135 patients and severe mutations of CFTR gene-F508del, W1282X and N1303K were found in a heterozygous state in 18 men, which amounted to 13,3%.

Mutations in the CFTR gene can cause some forms of azoospermia or oligozoospermia and can be inherited. It is known that among men with infertility, mutations in CFTR gene (encodes a special transmembrane regulatory protein of cystic fibrosis) and W1282X variant of CFTR gene lead to nucleotide change in 20 exon, leading to the formation of stop codons, and N1303K leads to the replacement of asparagine with lysine. The carrier frequency for individual mutations in CF gene in men with infertility is 12%. We identified the following mutations in the CFTR gene in men: delF508, W1282X, N1303K, which amounted to 6.9% of cases.

Levels of pro-inflammatory cytokines - IL-2, IL-6 and TNF-α in seminal fluid - were analyzed. The changes that we found in the studied cytokines were manifested by increased levels both in the first group and in the second group. According to the results of EIA, it was found that IL-2 level in the first group varied from 20 to 55 pg / ml with an average value of 49.6 ± 2.3 pg / ml. In the second group, this indicator was 2.3 times higher than the values of the control group and made up in averaged 56.8 ± 2.6 pg / ml ($P < 0.001$) with variability of data from 35 to 65 pg / ml.

According to several authors, IL-6 provides a rapid increase in the number of spermatogonia and differentiation of germ reproductive cells and Sertoli cells. In our studies, the level of IL-6 in the first group varied from 15 to 30 pg / ml. At the same time, the average value of IL-6 concentration in the group was 27.4 ± 1.2 pg / ml, which is 1.4 times lower than the control values ($P < 0.01$). In the second group, this indicator varied from 0 to 40.0 pg / ml with an average value of 18.6 ± 1.0 pg / ml, which was two times lower than the values of the control group, ($P<0,001$).

Finally, TNFα level in group 1 ranged from 35 to 120 pg / ml. At the same time, the average value of TNFα concentration in the group was 84.3 ± 2.2 pg / ml, which was 1.8 times higher than the data in the control group ($P < 0.01$). In group 2, this indicator varied from 50 to 135 pg / ml with an average value of 99.7 ± 2.4 pg / ml, which was 2.1 times higher than the control data, ($P<0,001$).

Thus, obtained data show that in men with CFTR gene polymorphism, the levels of studied cytokines in the seminal fluid significantly differ from those in men with infertility who do not have mutations in CFTR gene.

Conclusions.

Genetic factors are a common cause of gender formation anomalies and severe forms of infertility. In multifactorial pathology of reproduction, they play the role of predisposing factors to impaired fertility, determine the genetic background of reproductive health. Despite the importance of genetic factors in reproduction, medical and genetic examination of patients with childbirth problems remains insufficient. Introduction of new knowledge about the nature of genetically determined reproductive disorders will increase the efficiency of diagnostics, tactics for solving problems of reproduction and prevention of genetic diseases. Given the high frequency of genetic disorders, especially in men with severe forms of reproductive disorders, all patients with infertility should be advised to pass medical genetic examination. It also follows from the results of the conducted study that it is advisable to determine the levels of pro-inflammatory cytokines in complex, since any of the above factors alone does not give a complete picture of the nature of the taking place processes.

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