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Parameters Of Immunological And Molecular-Genetic Interrelation In Men With Impaired Fertility

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Relevance: To date, it has been established that the causes of male infertility are very diverse. Among the causes of male infertility are considered ejaculatory, sexual, anatomical changes in the structure of the genital organs, endocrine disorders, inflammatory processes, immunological factors, various disorders of spermatogenesis, environmental factors, and much more. In the structure of the causes of infertile marriage, male infertility occupies up to 40%, and it should be given the same close attention as female infertility.

It is known that genetic factors cause at least 30-50% of all cases of severe forms of infertility in men. Spermatogenesis is a complex biological process that depends on a precisely controlled cascade of activation and deactivation of certain genes. The result of the work of these genes is the process of maturation of spermatozoa from progenitor cells (spermatogonia). In humans, more than 2000 genes are involved in this process. Due to genetic disorders, various forms of infertility in their etiology and severity can occur: from minor violations of spermatogenesis to complete dysfunction of the gonads.

Among the gene factors associated with azoospermia, mutations/variants of the CFTR gene are the most common. They can cause cystic fibrosis (CF) and CBAVD syndrome (congenital bilateral aplasia of vas deferens), resulting in bilateral aplasia and obstruction of the vas deferens.

The study of the features of immunological reactivity in male infertility has not only a pronounced theoretical, but also practical interest. Of particular note is the lack of information about the state of the cytokine profile and its relationship with the parameters of immunity in infertility in men, as well as the impact of these disorders on the processes of spermatogenesis.

Target research - to study the characteristics of immunogenetic factors and their combinations that affect male reproductive function.

Materials and methods. On the basis of the Department of Urology and Andrology of TashIUV, 135 men aged 28 to 45 years with impaired fertility were examined.Standard spermiological, molecular genetic (CFTR gene polymorphisms) and immunological (IL levels-2, IL-6 and TNF-α)study. According to the results of immunogenetic studies to determine the CFTR gene polymorphism, 2 groups were formed: the 1st group - 117 men who did not have CFTR gene polymorphism and the 2nd group - 18 men with CFTR gene mutations (F508del, CFTRdele2,3 (21kb), 2143delT, 2184insA, G542X, W1282X, N1303K, 3849+10kbC>T). A molecular genetic study was performed on DNA isolated from peripheral blood lymphocytes by alcohol-salt treatment according to Miller. The

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CFTR gene was analyzed for the presence of the 8 most common mutations (F508del, CFTRdele2.3 (21kb), 2143delT, 2184insA, G542X, W1282X, N1303K, 3849+10kbC>T) allele by the specific RPS method using the Cystic Fibrosis test systems of NPO Litekh ".

The concentration of pro-inflammatory cytokines (IL-2, IL-6 and TNF-α) were carried out in the ejaculate by ELISA using the test kits "JSC Vector-Best" (St. Petersburg, Russia). The control group consisted of 20 practically healthy men who were married and had children.

Statistical analysis was performed using the Excel program from the Microsoft Office 2013 software package using Pearson's exact χ2 test. Differences were considered significant at a probability level of $p < 0.05$ andstandard programs (MS Excel 2002, Statistica 6.0). The degree of significance of differences between groups was assessed by the Fisher-Student test. Differences were considered statistically significant at $p<0.05$; $p<0.01$; $p<0.001$.

Results and its discussion. In order to study the features of the genetic contribution of the CFTR gene to the development of infertility in men, the 8 most common mutations of the CFTR gene associated with cystic fibrosis were analyzed in this study.Molecular genetic studies were carried out in 135 patients and severe mutations in the CFTR- gene were detected.F508del, W1282X andN1303K in the heterozygous state in 18 men, which was 13.3%.

The levels of pro-inflammatory cytokines - IL were analyzed-2, IL-6 and TNF-α in semen. The changes we found in the studied cytokines were manifested by an increased content, both in the 1st group and in the 2nd group.The level of IL-2 in group 1 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 averaged 56.8 ± 2.6 pg/ml ($P<0.001$) with data spread from 35 to 65 pg/ml.

The level of IL-6 in the 1st 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 -2nd group, this indicator varied from 0 to 40.0 pg/ml with an average value of 18.6 ± 1.0 pg/ml, which was 2 times lower than in the control group ($P<0.001$).

Finally, the level of TNFα 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 in the control group ($P<0.01$). In the 2nd group, 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 ($P<0.001$).

Findings: The data obtained showed that in men with the presence of CFTR gene polymorphism, the levels of the studied cytokines in the seminal fluid significantly differ from those men with infertility who do not have mutations in the CFTR gene. It also follows from the results of the study that it is advisable to determine the levels of pro-inflammatory cytokines in combination, since any of the above factors alone does not give a complete picture of the nature of the ongoing processes.

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