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CLINICAL AND IMMUNOGENETIC CHARACTERISTICS IN MEN WITH IMPACTED FERTILITY

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BACKGROUND

Immunogenetic disorders can be the cause of idiopathic male infertility. Mutations in the CFTR gene, involved in spermatogenesis or the formation of the male reproductive system, also in many cases cause impaired male fertility. It should be emphasized that there is no information about the state of the cytokine profile and its relationship with the parameters of immunity in infertility in men, as well as the influence of these disorders on the processes of spermatogenesis. **Objective:** The study involved 135 men with infertility, who were divided into 2 groups: a group of men without CFTR gene mutations and a group with CFTR gene mutations. **Result:** In the ejaculate, the level of proinflammatory cytokines - IL-2, IL-6 and TNFα - was determined. It was revealed that in men with the presence of CFTR gene polymorphism, the levels of the studied cytokines in the seminal fluid significantly differ from those in men with infertility who do not have mutations in the CFTR gene

KEYWORDS: male infertility, genetic research, cystic fibrosis, cytokines.

INTRODUCTION

For today, it has been established that the causes of male infertility are very diverse. Ejaculatory, sexual, anatomical changes in the structure of the genitals, endocrine disorders, inflammatory processes, immunological factors, various disorders of spermatogenesis, environmental factors and other factors are considered among the causes of male infertility. Today, in the structure of the reasons for infertile marriage, male infertility occupies up to 40%, and close attention should be paid to it as to female infertility.[1,3,8,10]

It is known that genetic factors are responsible for at least 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 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, forms of infertility, different in their etiology and severity, can occur: from minor violations of spermatogenesis to complete dysfunction of the gonads.[2,3,4,6,7]

Obstructive and non-obstructive form of azoospermia can be caused by various external causes (injuries, infections, vasectomy) or genetic factors. The most common are mutations/variants of CFTR gene among the gene factors, associated with azoospermia.[10] They can cause cystic fibrosis (CF) and CBAVD syndrome (congenital bilateral aplasia of vas deferens), 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, frequency of which in the Russian Federation is on average 1 per 10,000 people. CF is characterized by progressive clinical course and damage to the respiratory and digestive system. 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.[11] Characteristic spermatological signs of these disorders are azoospermia, oligospermia, pH<7, 0 and low level of fructose and a-glycosidase in ejaculate.[10] To solve the problem of childbearing for men with CF and CBAVD syndrome, biopsy of the testicles (which is effective in 85-90% of cases) is required, followed by IVF/ICSI procedure. Before carrying out programs of assisted reproductive technologies, these patients and their spouses need to undergo a medical genetic examination and counseling, in case of detection of pathogenic variants of CFTR gene in a spouse, these couples need to carry out preimplantation genetic testing of embryos for CF.[8,9,10,12,15]

CFTR Gene Mutations**.** Cystic Fibrosis Transmembrane conductance Regulator (CFTR) is a protein involved in the transport of chlorine ions through the cell membrane.

The gene, encoding this protein, has the same name. Presence of mutations in both copies of CFTR gene leads, as a rule, to the development of the most common hereditary autosomal recessive monogenic disease - cystic fibrosis, and can also be the cause of male infertility.[12,14] Human CFTR gene is located on the long arm of chromosome 7 in the area of q31. More than 900 different mutations of CFTR gene are known so far. About 70% of cases of cystic fibrosis are caused by the deletion of three base pairs, encoding amino acid phenylalanine in the 508th position of the transmembrane regulatory protein- delF508 (? F508). In addition, 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 study of the features of immunological reactivity in male infertility has not only a pronounced theoretical, but also practical interest. The lack of information on the state of the cytokine profile and its relationship with the parameters of immunity in male infertility, as well as the effect of these disorders on spermatogenesis processes, should be emphasized.[4,9,11]

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

MATERIALS AND METHODS

On the basis of the Department of Urology and Andrology of Tashkent Institute of Physicians Improvement, 135 men, aged 28 to 45 years with a diagnosis of primary infertility, were examined. Standard spermological, molecular- genetic (CFTR gene polymorphisms) and immunological (IL-2, IL-6 and TNF-α levels) studies were performed. 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 9 common mutations (F508del, CFTRdele2,3 (21kb), 2143delT, 2184insA, G542X, W1282X, N1303K, 3849+10kbC>T), making up totally about 77% of the total number of damaged chromosomes, as well as polymorphism IVS8-Tn.

Concentration of pro-inflammatory cytokines (IL-2, IL-6 and TNF-α) was carried out in the ejaculate by EIA method, using test kits of "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 Excel program from the Microsoft Office 2013 software package, using Pearson's chi-square ($\chi 2$) test. Differences in probability level $p < 0,05$ and standard programs (MS Excel 2002, Statistica 6,0) were considered significant. The degree of significance of differences between the groups was assessed by Fisher-Student test. Differences were considered statistically significant when $p<0,05$; $p<0,01$; $p<0,001$.

RESULTS AND THEIR 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. More detailed analysis is presented in Fig.1.

As we can see from the data of fig. 1, average age varies, depending on groups. 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.

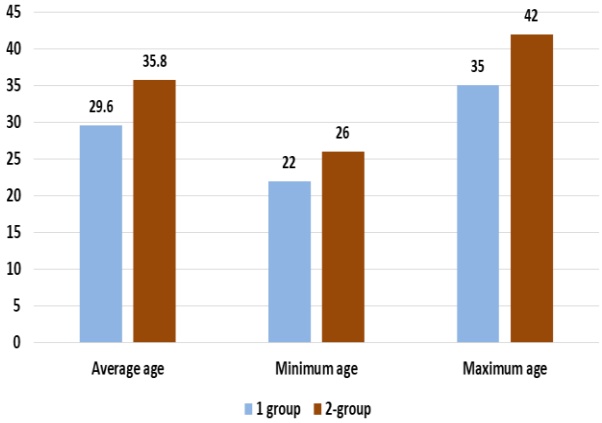

Fig.1. Age characteristics of the surveyed groups.

In the structure of pathozoospermia in men, both in the first and in the second group, asthenozoospermia prevailed, followed by: oligozoospermia, asthenoteratozoospermia, teratozoospermia and azoospermia. (table.1).

Table: 1.Forms of pathozoospermia in groups, (%).

Pathozoospermia	Men with infertility, n=135	
	1st group	2nd group
Asthenozoospermia	34 %	29 %
Oligozoospermia	23 %	22 %
Asthenoteratozoospermia	17 %	21%
Teratozoospermia	11%	9 %
Azoospermia	15 %	19 %

Previously, we carried out a population comparative analysis of four main CFTR gene polymorphisms: F508del; G542X; N1303K; W1282X. The results of our research have shown that F508del polymorphism in exon 10 has practical significance in the Uzbek population.
When conducting population comparison of the Uzbek population with representatives of the German, Romanian, Hungarian, Jewish, Brazilian, Colombian, Turkish populations on the prevalence of the risky allele of this polymorphism, statistically significant differences were revealed with all of them, except for Ashkenazi Jews and Turks.[16]

In order to study the features of the genetic contribution of CFTR gene to the development of infertility in men, this study analyzed eight the most frequent CFTR gene mutations, associated with cystic fibrosis. Molecular genetic studies were carried out in 135 patients and severe mutations of CFTR-F508del, W1282X and N1303K gene 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 (fig.2). 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.

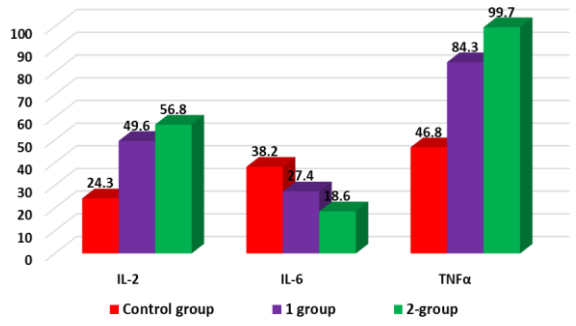

Fig: 2. The level of cytokines in the seminal fluid of the surveyed men, (pg/ml).

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 was 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 group2, 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.

CONCLUSION

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.

Currently, use of genomic technologies only comes into practice of examination of patients and couples with impaired reproduction. The prospects for their widespread use are undeniable. The introduction of new genetic knowledge about the nature of genetically conditioned reproductive disorders will improve the effectiveness of diagnosis, tactics to solve problems of reproduction and prevention of genetic diseases. Given the high incidence of genetic disorders, especially in men with severe reproductive disorders, genetic testing and counseling should be recommended for all patients with infertility. 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 occurring processes.

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