DIAGNOSIS OF GENETIC FORMS OF AZOOSPERMIA

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ABSTRACT — Aim: To identify the main genetic causes of azoospermia.
Methods. The study included 92 patients with azoospermia. In all patients we carried out genetic tests — karyotyping, PCR-diagnosis of blood.
Results. Genetic disorders were found in 35 (38%) men. Of these, the majority of men were with Kleinfelter syndrome — 21 (60%) and deletions in the AZF regions of the Y chromosome — 11 (31.4%).
Conclusions. Patients who have genetic abnormalities should receive comprehensive medical and genetic advice.

KEYWORDS — Male infertility, azoospermia, chromosomal abnormalities, Kleinfelter syndrome, AZF microdeletions

INTRODUCTION

Reduced sperm fertility occurs on average in 7% of men in the population [2, 7]. As a rule, laboratory and instrumental signs of male infertility are semen disorders that are quantitative and qualitative [2]. Male infertility is a polyetiological disease [1], which may be due to genetic factors. Genetic abnormalities are available azoospermia in 30-50% of patients [5, 6, 8].

It is known that there are several genetic causes of male infertility: chromosomal disruptions (Robertson translocations, structural changes in the karyotype, inversions); abnormal number of chromosomes (chromosomal disomy, development of Klinefelter syndrome); the appearance of fallen chromosomal sites (deletions) in the AZF locus of the Y chromosome; microdeletions and point mutations in the androgen receptor (AR) gene; abnormalities in the structure of sex chromosomes (local mutations) [3, 4].

Aim.

Identify the main genetic causes of azoospermia.

METHODS

The study included 92 male patients. All patients were aged 36±9 years. The study was carried out in accordance with the standards of good Clinical Practice (Good Clinical Practice) and the principles of the Helsinki Declaration. Inclusion criteria: absence of sperm in sperm (azoospermia), absence of pregnancy in the spouse for more than 1 year, consent to the processing of personal data. Exclusion criteria: female infertility factor, severe endocrine disorders, varicocele, cryptorchidism, sexually transmitted diseases. All patients underwent genetic examination — karyotyping, multiplex PCR with simultaneous amplification of several DNA fragments. Semen analysis was performed in accordance with WHO recommendations (2010). We determined the level of gonadotropins (follicle stimulating hormone (FSH) and luteinizing hormone (LH)) and testicular volume in all patients with genetic disorders. The examination of patients was performed using the test system Becman Coulter, Diagnostic System Laboratories (USA)

The statistical analysis was performed using spreadsheets "EXCEL" and "STATISTICA 6.0". The significance of differences between quantitative indicators was assessed using the Mann–Whitney test. Differences were considered significant at $p < 0.05$.

RESULTS

According to spermograms, azoospermia was recorded in all patients. We have not identified the genetic causes of the lack of germ cells in the sperm of 57(62%) men, and regarded these cases as idiopathic male infertility. The remaining 35 (38%) men had various genetic disorders. Of these, the majority were men with Klinefelter syndrome — 21 (60%). Microdeletions in the AZF regions of the Y chromosome were in 11 (31.4%) patients. The results of the genetic and clinical analysis (n = 36) of men with azoospermia are presented in Table 1. The average FSH level in men with genetically determined azoospermia of patients was 20.9±0.8 mIU/ml (Table 1). The average testicular volume was 10.25±1.2 ml. Those, these were patients with primary hypergonadotropic hypogonadism.

We recorded the highest fluctuations in FSH (21.2+1.4 IU/ml) in patients with Kleinfelter syndrome, and figure 1 shows a negative relationship between FSH levels and testicular volume in these patients.

Table 1. *The distribution of patients with azoospermia with a registered genetic pathology (n = 35)*

Results of genetic analysis		Number of patients n(%)	testicular volume (ml)	FSH level (IU/ml)
Kleinfelter syndrome (n=21)	47XXY	11 (31,4%)	8,2+0,8*	24,2+2,8*
	47XYY	4(11,4%)	10,4+0,6*	21,5+1,9*
	46XY(mosaicism)	6 (17,1%)	12+1,3*	18,1+2,1*
deletions in AZF regions (n=11)	AZFa	2(5,7%)	6,2+1,2*	24,8+2,4*
	AZFb	6(17,1)	7,0+1,4*	23,0+2,9*
	AZFc	3(8,5%)	11,0+0,9*	19,2+2,7
CFTR (Cystic fibrosis transmembrane conductance regulator)		2 (5,7%)	18,2+2,2	14,1+2,5*
AR (mutations in the androgen receptor gene)		1(2,8%)	9,0+1,4*	22,8+3,0*

** at p <0.05 when comparing testicular volume and FSH level.*

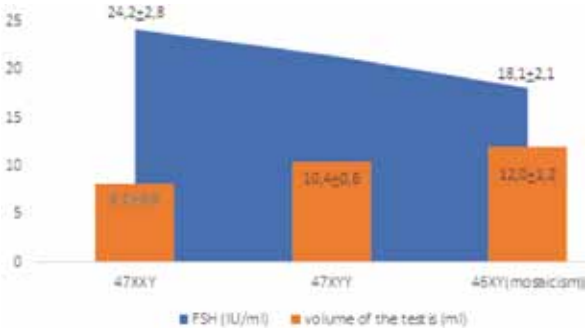

Fig. 1. *The dependence of FSH level and testicular volume in the Klinefelter syndrome*

DISCUSSION

Diagnosis of genetic abnormalities is extremely important in the examination of patients with male infertility in order to assess the success of assisted reproductive technologies (ART) [2, 7]. Syndromic genetic mutations are often the cause of azoospermia. The syndromic forms of pathospermia have a characteristic phenotype, so the recognition of this disease is not difficult even at the stage of primary diagnosis of male infertility. However, with microstructural genetic rearrangements, for example, with Kleinfelter syndrome (mosaic form), primary diagnosis is difficult because the phenotypic visualization of the patient is complicated [4]. Therefore, medical and genetic consultation in azoospermia should be performed necessarily to each patient.

Microdeletions of the Y chromosome are the cause of azoospermia in 15% of cases [7]. Identification of sub-regions of microdels of AZF region of the Y chromosome allows to evaluate personal prospects of treatment of male infertility, as microdeletions of AZFa, AZFb or AZFa/b are associated with the worst prognosis of testicular sperm production for ART programs [4].

In our study, most of the genetic abnormalities associated with azoospermia were represented by Kleinfelter's syndrome — 21(22.8%). Deletions in the region of the AZF of the Y-chromosome gene were detected in 11(11.9%) people. We believe that the indications for genetic research in male infertility are: azoospermia; in the presence of several unsuccessful attempts of art; in the case of habitual miscarriage of the fetus in the first trimester of pregnancy; in the presence of family relatives with congenital defects.

CONCLUSIONS

Genetic male infertility is associated with mutation of chromosomes, change in their number and violation of the structure. In men with severe infertility (azoospermia) genetic abnormalities occur in every third case.

Patients who are suspected of having genetic abnormalities should receive a comprehensive genetic examination before selecting assisted reproductive techniques that can reduce the potential risk of transmission of genetic aberrations to offspring. Given that there is a high percentage of men with idiopathic azoospermia, for this category of patients it is necessary to conduct additional genomic research (exoma sequencing and chromosomal micromatric analysis).

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