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Genomic and Chromosome Mutations in Complex with Environmental and Lifestyle Factors as Reasons for Azoospermia and Oligoasthenoteratozoospermia

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Abstract. The aim of the present study was to investigate and characterize the manifestation of genomic and chromosomal mutations in complex with the environmental and lifestyle factors as reasons for azoospermia and oligoasthenoteratozoospermia by applying a classical cytogenetic assay and questionnaire survey. 1540 men were included in the survey. By conventional sperm analysis, 183 of them were diagnosed with azoospermia and oligoasthenoteratozoospermia. Based on the cytogenetic analysis, it was concluded that trisomies, in particular Klinefelter syndrome, and structural chromosome aberrations such as translocations and chromatid fragmentation, are directly related to male infertility. Together with harmful habits such as smoking and alcohol use, they are among the major causes of azoospermia and oligoasthenoteratozoospermia. The results obtained could be successfully used in the implementation of a system of activities for the prophylaxis of male reproductive health.

Key words: mutations, environmental and lifestyle factors, male infertility.

Introduction

Human infertility affects between 10% and 15% of couples in reproductive age and male infertility has been associated with approximately half of these problems (YU *et al.*, 2014). Various genetic causes, as well as environmental and lifestyle factors could disturb the course of gametogenesis and the functions of the gametes and to affect the male fertility (SHARPE & IRVINE, 2004; KRAUSZ, 2008; MATZUK & LAMB, 2008; OATES, 2008; GUDELOGLU & PAREKATTIL, 2013). Among them, the role of DNA fragmentations in spermatozoa nuclei (SAKKAS *et al.*, 1999; BENCHAIB *et al.*, 2003; EVENSON & WIXON, 2005; SMITH *et al.*, 2006; YU *et al.*, 2014), as well as the occurrence of genomic mutations, some of which directly associated with male infertility (LANFRANCO *et al.*, 2004; VISOOTSAK & GRAHAM, 2006) have been investigated and analyzed.

In Bulgaria, the relationship between chromosomal diseases and fertility disorders in men is slightly studied. There are incomplete data on the occurrence of Klinefelter syndrome in the Bulgarian population, as well as the relationships between other possible chromosomal aberrations and male

reproductive health, and in particular, the associations of chromosomal mutations with azoospermia and oligoasthenoteratozoospermia as the most severe conditions of male infertility (DZHOGLOV & IVANOVA, 2016; LINEV *et al.*, 2017).

Therefore, the aim of the present study was to investigate and characterize the manifestation of chromosomal mutations in patients with azoospermia and oligoasthenoteratozoospermia by applying a classical cytogenetic assay.

Materials and Methods

This investigation was done accordingly to ethical principles (Institutional Ethics Committee Certificate N 2/16.01.2019). Informed consent was obtained from each participant entering the study. The study included data from 1540 men (mean ± SD age 33 ± 7.4 years) who visited a reproductive health office in Plovdiv for prophylaxis or for a reproductive problem. All participants have voluntary given answers to a questionnaire providing data on medical history, environmental and lifestyle factors, as well as standardized semen samples.

Among the men with oligoasthenoteratozoospermia and azoospermia (totally 183 - 14.1%) 17 patients were subjected to cytogenetic analysis. The following was found for them:

- excessive increase of the boy or man height that is not a family feature;
- abnormalities of external genital organs or anomalies during puberty period;
- reproductive failures (TONCHEVA *et al.*, 2014).

Venous heparinized blood was used for purposes of cytogenetic analysis. Chromosomal preparations were made after cultivating of 72-hour lymphocyte culture. Hypotonic treatment and fixation in Clarke fixation (methanol:glacial acetic acid – 3:1 ratio) was done after the blocking of cell division with cytostatics. In order to denature the chromosomal protein, enzyme (trypsin) or temperature treatment was accomplished before the G-Banding staining. In the course of microscopic investigating Carl Zeiss - Laboval 4 and Olympus BX - 40 microscopes were used. After photograding of quality metaphase plates a karyotype analysis was performed (VALKOVA, 1999).

Descriptive statistics was used to characterize the frequency of the groups compared.

Results and Discussion

In the whole group of the participants (1540), a diagnosis of oligoasthenoteratozoospermia was established for 96 (7.4%) of them, and azoospermia – for 87 (6.7%).

Data from the survey showed that 15.8% of men who suffer from azoospermia and oligoasthenoteratozoospermia were smokers, 10.9% were alcohol users, 8.2% are receiving medication, 5.5% were working in a harmful professional environment, 4.9% have taken anabolic steroids and 1.1% - drugs.

The conclusions based on the conventional sperm analysis showed that 11 of the participants included in the cytogenetic investigation have diagnosis azoospermia (lack of spermatozoa in the ejaculate), and the other 6 men – a severe form of oligoasthenoteratozoospermia (violation of the three variables – spermatozoa concentration, motility and normal morphology).

Data from the cytogenetic study of the 17 participants indicated that 12 (70.6%) of them have had a normal male karyotype. Among the remaining 5 (29.4%), three cases of Klinefelter syndrome (17.6%), one case of balanced translocation 13/21 (5.9%) and one case of chromatid rupture with dislocations (5.9%) were found - Fig. 1.

Men with Klinefelter syndrome refer to the group of patients with azoospermia and those with structural chromosomal aberrations (translocation and chromatid fragmentation) - to the group of patients with oligoasthenoteratozoospermia.

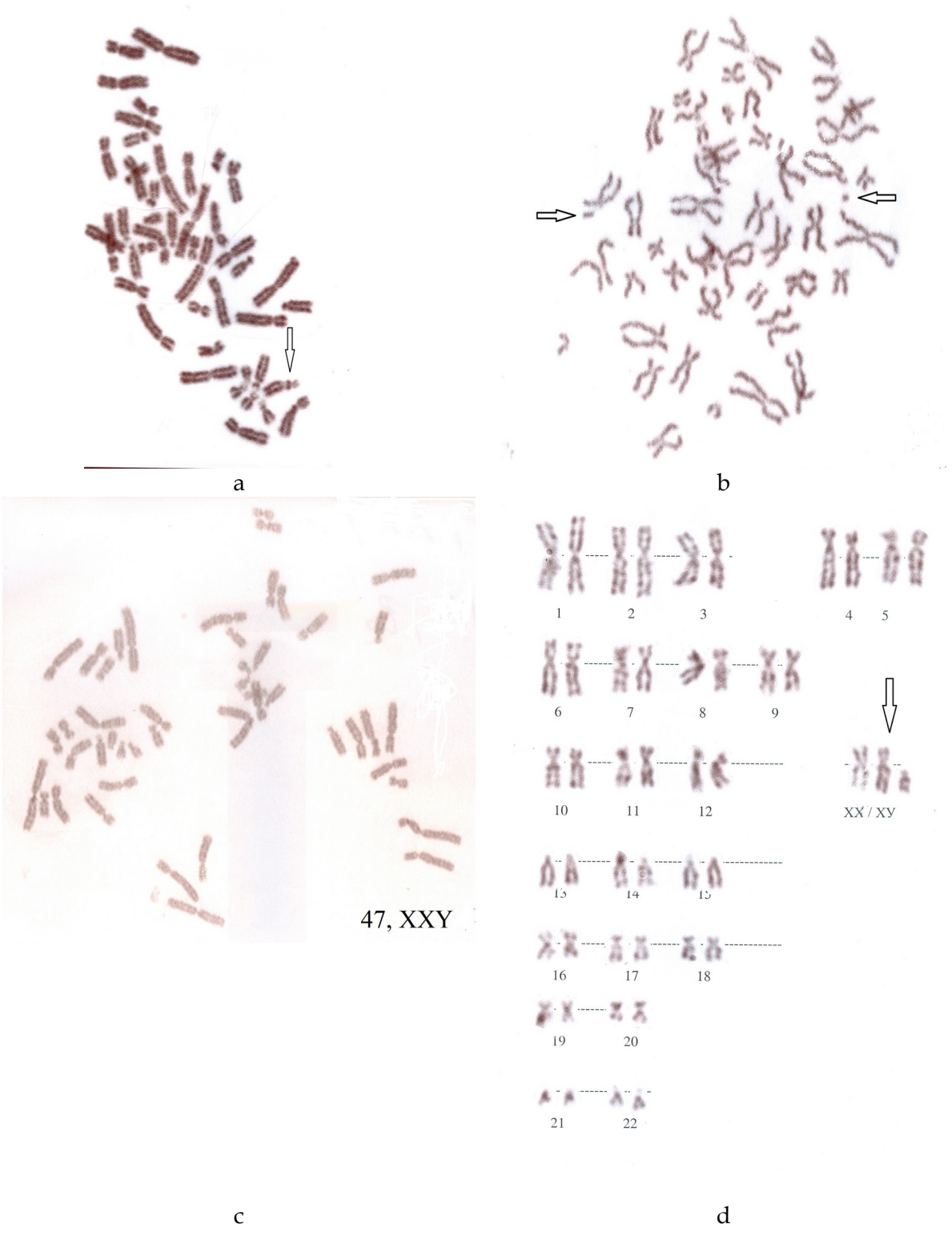

Fig. 1. Chromosomal and genomic abnormalities: a) translocation 45, XY, t (13; 21); b) chromatid fragmentation with dislocation; c) Kleinfelter syndrome (47, XXY) – metaphase plate; d) Kleinfelter syndrome (47, XXY) – karyogram.

In studying problems related to male reproductive health, aneuploidy, leading to an imbalance in the karyotype, has an important clinical significance (TONCHEVA *et al.*, 2014).

The genomic mutation most commonly associated with male infertility is "polysomy X (+ Y)," known as Klinefelter syndrome. Various variants of the syndrome have been described but the most common karyotypes of this group are 47, XXY and 48, XXXY. Its frequency is between 0.1% and 0.2% in the general population and may reach to 3.1% in populations with a higher rate of infertility (GUDELOGLU & PAREKATTIL, 2013).

The results of this study indicate a similar frequency of 0.19% within the general population studied and a frequency among the men with azoospermia – 3.45%, which is significantly lower than in the reported by LINEV *et al.* (2017) results for men with azoospermia, among whom a classic version of Klinefelter syndrome was 10.26%.

Structural aberrations of autosomes are much more common in infertile men than in the general population. There was found, that the Robertsonian translocations are 9 times more common in infertility patients than in the general population and also that reciprocal translocations are 4 to 10 times more common in infertile than in fertile men. The most common Robertsonian translocation associated with male infertility is found to be between chromosomes 13 and 14 of the human karyotype (O'FLYN O'BRIEN *et al.*, 2010).

Data of the present study showed that men with Klinefelter syndrome refer to the group of patients with azoospermia and those with found structural chromosome aberrations – to the group of patients with oligoastenoteratozoospermia. In both cases – azoospermia and oligoasthenoteratozoospermia – the relationship between the established mutation (genomic or chromosomal) and male infertility is obvious.

In terms of translocations and their relationship to male infertility, the data from our study supports the findings of other authors (FERLIN *et al.*, 1998; O'FLYN O'BRIEN *et al.*, 2010), with the difference that in our findings translocation is not between 13 and 14, but between chromosomes 13 and 21. The results suggest that structural aberrations affecting the chromosome 13 probably correlate with male infertility, regardless of whether they are translocation exchanges of the chromosome 13 with 14 or 21.

Our earlier studies (DZHOGLOV & IVANOVA, 2016) on the azoospermia showed that for men with Klinefelter syndrome the mean volume of the ejaculate is 0.64 ml less than in the other patients. There was also found a statistically significant correlation between the volume of ejaculate and smoking – the smokers emit an average of 0.61 ml less ejaculate than non-smokers.

It has been also found that in the group of smokers the volume of ejaculate is increased by increasing the number of smoked cigarettes per day (DZHOGLOV & IVANOVA, 2016). The authors associate this with the increased production of seminal vesicles and secretions of prostate gland as an expression of local exudative vascular reaction – a result of the intense smoking. A similar trend has been established for another risk factor – alcohol. No significant difference in the ejaculate volume has been observed between drug users and those who do not use drugs (DZHOGLOV & IVANOVA, 2016).

Conclusions

Based on the cytogenetic analysis, it could be concluded that trisomies, in particular Klinefelter syndrome, and structural chromosome aberrations such as translocations and chromatid fragmentation, are directly related to male infertility. Together with harmful habits such as smoking and alcohol use, they are among the major causes of azoospermia and oligoasthenoteratozoospermia. The results obtained could be successfully used in the implementation of a system of activities for the prophylaxis of male reproductive health.

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