Cytogenetic Abnormalities Found in Patients with Reproductive Problems

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ABSTRACT
Introduction: One of the important causes of male infertility is aberration at the chromosomes. Aim: The main purpose of this study was to determine the frequency and types of chromosomal aberration in infertile/sterile men whose samples were analyzed in the Center for Cytogenetics of Faculty of Medicine University of Sarajevo in the last four years. Methods: A total of 353 infertile/sterile men, between the ages of 22-55 years, referred for cytogenetic analysis to the Center for Genetics of Faculty of Medicine during the period 2013-2016. Karyotyping was performed on peripheral blood lymphocytes by using the Giemsa trypsin banding (GTG) technique. Results: The structural and numerical chromosomal aberration in infertility/sterility of men found with the incidence of 6% (20/353). Out of the 20 patients with abnormal cytogenetic diagnosis, structural chromosome abnormalities were observed in 17 (85%) patients and 3 (15%) with numerical aberrations. The type of aberrations mostly found were Robertsonian and reciprocal translocations (35%, 35%, respectively). Conclusions: The incidence of chromosomal abnormalities in infertile/sterile males suggests that the cytogenetic analysis is an important in male infertility, especially if it will be used for the purpose of assisted reproduction techniques.

Keywords: Cytogenetic analysis, structural and numerical chromosomal aberration, infertility.

1. INTRODUCTION
The World Health Organization has described infertility as a health problem of global concern. Infertility is defined as inability to conceive in period of 12 months of normal intercourse (without contraception) (1). Infertility affects about 10%-15% of couples around the world (2). Genetic factors have only 10% of influence at infertility at men. There are many and diverse causes of male infertility, including accidental causes, hormonal imbalance, erectile dysfunction, infections, antisperm antibodies, environmental pollutants, or genetic factors. (3, 4). However, infertility causes are unknown in 12%-41% of men (5). Chromosomal anomalies are considered as one of the most important causes of male infertility. The incidence of chromosomal abnormalities is about 1:1000, half of them are numerical, and half of them are structural aberrations, but also, both normalities can occurs together. Infertility caused by numerical and structural aberrations occurs more often in male than in female. The rate of chromosomal abnormality in infertile men is 2.1-19.48% (6). Chromosomal abnormalities are not the only genetic causes of male infertility. Deletion of the Y chromosome region containing the azoospermia factor (AZF) is considered the most common genetic cause of male infertility (7).

2. MATERIAL AND METHODS
Sample
The cross-sectional study included cytogenetic analysis of 353 male that has been performed in Center for Genetics of Faculty of Medicine, University of Sarajevo. The mean age of patients was 35 years (22-55). Participants recruited in the study had already been considered as an infertile/sterile or whose spouse had more than one spontaneous abortion. The present study was performed in accordance with the ethical standards and the Declaration of Helsinki.

Methods
Cultivation of peripheral blood sample at patients’ is performed by...
short-term cultivation methods (8). Cytogenetic analysis is performed under International System of Human Chromosomal Nomenclature (ISCN) regulations (9). With every karyotype analysis 25 cells per sample is used by G-banding technique (10). C-bend is used to confirm heterochromatic regions.

Statistical analysis was done using -SPSS version 19.0. (Chicago, IL, USA) program. Data were expressed in term of frequency such as total number of cases and percentage of total number. The average age of the subjects age was presented as median with the range of minimum and maximum of the variable.

3. RESULTS

Cytogenetic analysis showed that among the 353 patients included in the study, 333 men (94%) had a normal karyotype (46; XY), and 20 men (6%) had various chromosomal abnormalities. Analysis of chromosomal abnormalities in 20 patients (the mean age was 34 (27-43) years) revealed (85%) abnormality of autosomal chromosomes and (15%) of sex chromosomes. They were dominantly infertile and only 1% sterile. Average life expectancy at men with chromosomal aberrations is 34 years and without chromosomal aberrations 35 years.

The most frequent structural aberrations were Robertsonian translocation (Figure1) and reciprocal translocation (7, 7 cases, respectively) followed by inversion of chromosome 9 detected in three cases. Numerical aberrations were detected in three cases. A balanced translocations rarely cause an abnormal phenotype (16). Pericentric inversion of chromosome 9 can cause clinical problems on offspring of the inversion carrier, as well as at infertility of unknown etiology sex linked and it be a cause of the disturbances of spermatogenesis (17, 18).

In the present study, we found balanced translocation to be the most frequent structural aberration among these patients. A balanced translocations rarely cause an abortion, their determination is very important. Also, it is cause of offspring conception with unbalanced karyotype of sex chromosomes (11), while in our case there were more changes on autosomes (85% vs. 15%).

The most common autosomal abnormality detected in infertile men was the Robertsonian translocation karyotype. Among them the most frequent was 45,XY,t (13;14) karyotype and only one with 45,XY,t (21;21) karyotype were detected in our study.

The most principal role in male infertility is performed by numerical and structural chromosome abnormalities.

Normal chromosome pairing and segregation at meiosis I (formation of unbalanced gametes and subsequent unbalanced abnormal offspring) is influenced by autosomal abnormalities (12). Low sperm concentrations and abnormal sperms with male infertility and increased miscarriages can be caused by structural chromosome autosomal abnormalities (13).

Both the chromosomes involved in translocation and the location of the breakpoints are likely to be determining factors for the fertility status of the patient. In addition, Robertsonian translocation can result in offspring with Down syndrome or Patau’s syndrome or in gestational loss of concepts with monosomy of chromosome 13, 14 or 21, or trisomy of chromosome 14, which are lethal (14). Male infertility and chromosomal anomalies are often closely related and reciprocal translocations are the most frequent (1 in 600) structural chromosomal anomalies in humans (12). Carriers of these translocations normally do not have any abnormal phenotype which leads to having normal offspring, recurrent miscarriage also chromosomally abnormal offspring and in some cases infertility (15).

In the present study results implicate the contribution of structural and numerical chromosomal aberration in fertility/sterility of men. According to updated and published studies, the usual cause of infertility is aneuploidy of sex chromosomes (11), while in our case there were more changes on autosomes (85% vs. 15%).

Table 1. Type and frequency of chromosomal aberration in infertile/sterele man. Legend: Data are presented as absolute number and percentage of total number of cases affected by a particular chromosome aberration. n-number of cases

| Type of aberrations | Karyotype | Frequency(n) | Percentage(%) |
|---------------------|-----------|--------------|---------------|
| Structural aberrations | 47,XY+marker | 1 | 5% |
|                     | 46,XY/45,X | 1 | 5% |
|                     | 47,XXY     | 1 | 5% |
|                     | 46,XY, inv9(p11;q13) | 3 | 15% |
|                     | 45,XY,robl3;14(q10;q10) | 6 | 30% |
|                     | 46,XY,t(16;7)(q25;q36) | 1 | 5% |
|                     | 46,XY, der5,t(5;19)(q35;p13.22) | 1 | 5% |
|                     | 46,XY, t(11;17)(q13.3;q25) | 1 | 5% |
|                     | 46,XY,t(5;9)(p15;q22) | 1 | 5% |
|                     | 46,XY,t(14p;q11q) | 1 | 5% |
|                     | 46,XY, der 12, t(12q;18q) | 1 | 5% |
|                     | 45,XY,robl21,t(21;21)(q10;q10) | 1 | 5% |
|                     | 46,XY, der20, t(13q14-34;20q13) | 1 | 5% |

Figure 1: Karyotype of a patient with Robertsonian translocation: 45,XY,t(13;14) (q10;q10)
5. CONCLUSION

This cytogenetic study on infertile or sterile men confirmed previous reports that chromosomal abnormalities might be one of the most common causes of infertility and a risk for transmission of these genetic defects to the future generations. Therefore routine cytogenetic analysis are necessary prior to reproductive techniques.

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