Original Research Article

Chromosomal aberrations in couples with infertility

Abstract

Aims: : One of the most significant causes of infertility in men and women are chromosomal abnormalities. There is a growing trend of postponing family planning so that couples want to have children in the later years of reproductive age, more precisely in the middle or late thirties. For this reason, we aimed to investigate the age, frequency, and types of significant cytogenetic abnormalities among infertile couples.

Study design: Retrospective study.

Place and Duration of Study: 142 couples (284 patients) were referred to the Center for Genetics of the Faculty of Medicine in Sarajevo between 2018 - 2022.

Methodology: The research included 284 respondents aged 20 to 54 with infertility. Karyotyping was performed on peripheral blood lymphocytes using the Giemsa trypsin banding (GTG) technique.

Results: The highest frequency of infertility was observed in the couples group aged 30-39 years. Chromosomal aberrations were found in 8 (2.8%) couples with infertility. Out of 160 cases of primary infertility, aberrant karyotypes were recorded in five patients, and three aberrant karyotypes were recorded out of 124 patients diagnosed with secondary infertility. Compared to numerical aberration, the most common type of chromosome was a structural aberration. In both types of infertility, a structural aberration of chromosome 9 inversion was recorded.

Conclusion: The frequency of chromosomal abnormalities and the age of couples with infertility suggest that cytogenetic analysis is essential for the timely detection of the infertility cause. It has special significance for couples who decide to have assisted fertilization.

Keywords: Infertility, structural and numerical chromosomal aberration, age.

1. INTRODUCTION

According to the World Health Organization, infertility is one of the most significant health problems. Infertility is defined as the inability to achieve pregnancy within a year of regular sexual intercourse (without the use of contraceptives). Infertility can be primary or secondary. Primary infertility is when pregnancy has never been achieved, and secondary infertility is when at least one prior pregnancy has been achieved. The reason for infertility can be in both men and women, and sometimes it is impossible to explain the causes. Estimates show that 48 million couples and 186 million individuals live with infertility (1). In men, infertility is most often caused by problems in ejaculation (2), a lack or low level of sperm, or abnormal shape (morphology) and movement (motility) of sperm. In women, infertility can be caused by several abnormalities of the ovaries, uterus, fallopian tubes, and endocrine system, among others. Some of the reasons are genetic, endocrine, physiological, anatomical, and immunological abnormalities of the reproductive system, which can affect the odds that a woman will achieve pregnancy and deliver a healthy and alive child (3). Genetic causes of infertility can be

numerical and structural chromosomal aberrations affecting either autosomal or sex chromosomes and due to either monogenic or polygenic disorders. Determining the exact cause of infertility is important because chromosomal aberrations are responsible for 2-14% of male infertility (4) and as much as 10% of female infertility (5). Regardless of the cause of the problem, it is very important to detect the problem in time. Assessing the risk of transmission is essential in cases where genetic disorders are behind such problems. Detecting a chromosome or gene change allows the possibility of providing precise genetic information about inheritance risks (6).

2. MATERIAL AND METHODS

In this retrospective/cross-sectional study, we analyzed the cytogenetic results of 142 couples (284 patients) referred to the Center for Genetics of the Faculty of Medicine in Sarajevo between 2018 – 2022 for infertility analysis purposes. The research included 284 respondents aged between 20 to 54 age. None of the enrolled patients had clinical or laboratory abnormalities that could cause primary or secondary infertility.

When arriving at the Center for Genetics at the Faculty of Medicine in Sarajevo, couples were informed about the goals and diagnostic potential of the analysis that were to be performed. The Ethical standards and the Declaration of Helsinki were performed in the present study.

Cytogenetic analyses were performed on peripheral lymphocytes stimulated with phytohemagglutinin and cultured using standard techniques (7). A commercial medium (*PB–MAX™ Karyotyping* medium (*Gibco*)) cultivated peripheral blood. With every karyotype analysis, 25 cell term samples were used by the G-bending technique (between 550 and 850 visible bands). Microscopic examination at 400x magnification using Olympus BX53 microscope (Germany), with CytoVision software (CytoVision, AB Imaging, Germany). Instructions and rules given by the International System of Human Chromosomal Nomenclature (ISCN) were followed when the cytogenetic analysis were performed (8). With every karyotype analysis, 25 cells terms samples were used by the G-bending technique (9). The results were analyzed using SPSS 20 (*Statistical Package for the Social Sciences*, IBM, NY, USA) program. Data were expressed in frequency, such as the total number of cases and percentage of the total number. The average age of the subject was presented as the median with the minimum and maximum range of the variable.

3. RESULTS

In the framework of our study, 142 couples, or 284 patients referred to the Center for Genetics due to some reproductive issues, were examined. Reproductive problems concern infertility, primary and secondary. The lower age limit of our population was 20, and the upper age was 54. The mean value was 34.80, with a standard deviation of ± 5.94.

Most patients belong to the 30-39 age category (Figure 1), with a total of 173 (61%). Within the group of patients aged 40-49, there are 56 patients (19%), 52 (18%) were in the 20-29 age group, and only three patients (1%) belonged to the 50 and over age group.

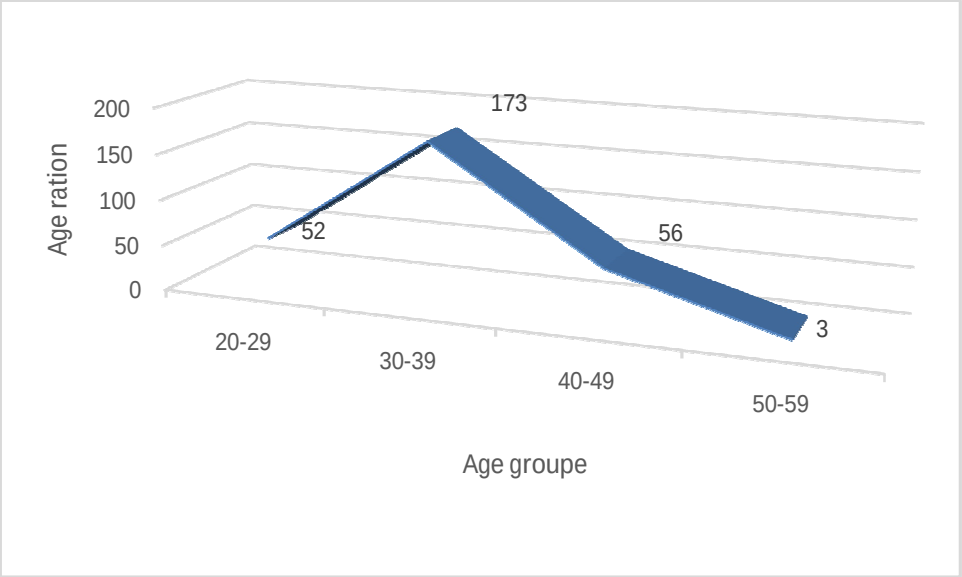

Figure 1. Age distribution of couples with infertility

Most men were in the 30-39 age range, 86 (60.5%) of them, followed by those in the 40-49 age group. There were 35 (24.6%) respondents in this group, and 18 (12.7%) men were from the 20 to 29 age group. The smallest number of respondents belong to the 50-59 group, with only three (2.1%).

Regarding women, most of them were in the age group from 30 to 39 years, 87 (61.3%). Then, 34 (24%) belonged to the 20-29 age group, and 21(14.7%) women were in the 40-49 age group.

Of 284 patients, 160 were assigned to the primary infertility group (56,4%). One hundred twenty-four patients (43,6%) were diagnosed with secondary infertility. Out of these patients, five women had three spontaneous abortions, while the other had one or two spontaneous abortions, and none of the total numbers achieved healthy delivery upon pregnancy.

Eight cases with the aberrant karyotype were recorded in couples with infertility. Out of 160 cases of primary infertility, aberrant karyotypes were recorded in five patients (3.1%), and three aberrant karyotypes were recorded out of 124 patients diagnosed with secondary infertility (2.4%). Table 1. shows the karyotype type of infertility, aberrations, age, and gender. The most common was the structural change of inversion chromosome 9 (25%).

Table 1. Type chromosomal aberration in infertile couples

Karyotype	Gender	Age	Type of infertility	Type of aberrations
46,XX, inv(9)(p11;q13)	female	29	Primary	Structural
46,XX, inv(9)(p11;q13)	female	31	Primary	Structural
46,XY, inv(9)(p11;q13)	male	27	Primary	Structural
46, XY, t(3;11)(q29;q14)	male	29	Primary	Structural
46,XY,t(4;21)(q12;q11.2)	male	31	Primary	Structural
46,XX, inv(9)(p11;q13)	female	38	Secondary	Structural
46,XX(67)/47,XXX(33)	female	30	Secondary	Numerical
45,XX,rob(13;14)(q10;q10)	female	32	Secondary	Numerical

The mean age for subjects with the aberrant karyotype was 31 years, with a standard deviation of ±7.57. The mean value for 3 male respondents in this group was 29 years, with a standard deviation of ± 5.6. The mean age of 5 female respondents in this group was 32 years, with a standard deviation of ± 2.12.

A t-test was also performed for two groups of subjects (male and female as part of the population with aberrations) to determine whether there was a statistically significant difference in age between the subjects of the two sexes. The *P* = 0.3868, so this data is taken as an argument that there is no statistically significant difference regarding the age of male and female subjects with present chromosomal aberrations. There was also no statistically significant difference in the frequency of chromosomal aberrations between men and women.

Aberrations were found in three women with secondary infertility. Two women with numerical chromosomal abnormalities had two spontaneous abortions, while one with inv(9) had only one spontaneous abortion.

DISCUSSION

Millions worldwide have infertility problems. Many causes affect infertility, including genetic factors (10, 11). Chromosomal aberrations represent one of the most significant causes of infertility and spontaneous abortions. The frequency of aberrant karyotypes in the general population ranges from 0.5% to 1%, while this percentage is significantly higher in people with reproductive problems (12). In this research, the percentage with an aberrant karyotype is 3.1% in people with infertility, which is a large percentage compared to the number of subjects. Some pregnancies, unfortunately, end in spontaneous abortion. One of the causes of spontaneous abortions is chromosomal anomalies, mostly aneuploidy (13, 14). Interestingly, we recorded the exact structural change, inversion of chromosome 9, in women with primary and secondary infertility. A man also had this inversion. Regarding the pericentric inversion of chromosome 9 (inv(9)), we have divided opinions among scientists. According to some authors, this inv(9) is considered the standard variant, and according to others, this type of inversion affects infertility (15,16).

In this study, only one woman had an aberration on the sex chromosome X, while other patients had aberrations on the autosomal chromosomes. The karyotype of that woman is mosaicism 46XX/47XXX. She had more cells with 46, XX (63) than triple X (37), so she became pregnant after several years of marriage but unfortunately had a miscarriage. Triple X is a rare chromosomal abnormality in approximately one in 1000 female births. This syndrome is discovered in adulthood in some women, most often due to fertility problems (17). As was the case with our patient, who discovered that she had triple X syndrome only at 30.

The frequency of chromosomal aberrations among couples with recurrent, repeated miscarriages (two or more) varies from 2-8% (18). In this research, cases with one, two, or three repeated abortions were recorded. Interestingly, chromosomal aberrations were not recorded in couples with three habitual abortions. The literature shows data on the increased frequency of chromosomal aberrations in people with more spontaneous abortions. Research conducted by Kiss et al. presents 108 couples (216 individuals) with a history of frequent spontaneous disorders, with a recorded frequency of chromosomal aberrations of 5% in couples with two miscarriages, 10.3% in couples with three miscarriages, and a frequency of 14.3% in couples with four or more miscarriages. (19).

The frequency of chromosomal aberrations in the general population ranges from 0.37%-1.86%, while this percentage in people with infertility is 3.95%-14.3% (20).. In couples with secondary infertility, out of 124 patients, chromosomal aberrations were found in three women. Those three women had spontaneous abortions.

According to some authors, chromosomal aberrations are more frequent in women with secondary infertility than in women with primary infertility (21, 22). However, according to research by scientists Liu et al., the

frequency of chromosomal aberrations is higher in persons with primary infertility and was 9.29% (800/8606), in contrast to those with secondary infertility 5.47% (285/5213) (20). These studies confirm an increased frequency of chromosomal aberrations in women with infertility (21, 23). Due to the small number of samples, this study did not show a difference in the frequency of chromosomal aberrations in women with primary or secondary infertility. There is no statistically significant difference in the frequency of chromosomal aberrations between the male and female populations in this study. A total of 3 chromosomal aberrations were recorded in men (5%; 5/100) and five aberrations in women (6%; 6/100). According to research by Benchikh et al., the percentage of chromosomal rearrangements is higher in men with reproductive problems than in women. In our previous research, more men had reproductive problems caused by chromosomal aberrations (24).

There is a growing trend of postponing family planning so that couples want to have children in the later years of reproductive age, precisely in the middle or late thirties. A woman's age is recognized as the main limiting factor of fertility and good reproductive success. The trend of older parents applies not only to women but also to men. For example, 19/1000 pregnancies in Germany were in the 40-44 age group in 1991. This rate increased to 61/1000 births in 2013 (25). This research showed that the number of couples over 40 (20%) who underwent cytogenetic analysis also increased. Due to a lack of natural conception, most couples decided to in vitro fertilization. They did not want to have children earlier because of their careers and living conditions. Given the increasing worldwide rate of women of advanced childbearing age over recent decades, increasing attention is paid to paternal influence on reproductive success (26). Premature menopause in the reproductive age and the increased prevalence of deliberately delaying pregnancy in developed countries contribute to female infertility (27, 28).

Given that more and more couples with reproductive problems are recently deciding on assisted reproduction (ART), it is very important to determine the cause of infertility (29). Especially when it comes to Robertsonian translocation (RT), people with RT are peaceful carriers of a balanced translocation and are primarily free of phenotypically manifested anomalies. At the same time, this aberration is detected most often due to reproductive problems. Their offspring are endangered to varying degrees depending on whether the translocation occurred between homologous or non-homologous chromosomes. Offsprings of people with RT can have the same balanced translocation but also an unbalanced one. In that case, the karyotype of the child would be either trisomy or monosomy. This is why cytogenetic analysis, as well as genetic counseling, provides adequate guidance and information about the existing risks when it comes to infertility treatment and the benefits and possible outcomes thereof (30).

4. CONCLUSION

Cytogenetic testing of couples with reproductive problems can help discover the cause of infertility. Finding the cause of infertility in men and women is essential for genetic counseling, especially in cases where couples decide on assisted fertilization.

Consent

As per international standard or university standard, patients' written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

"All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki."

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