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RESEARCH ARTICLE

Spectrum of Chromosomal Abnormality among 84 Indian Infertile Couples

Puspal De[1], Jayasmita Mahapatra[2], Pranay Gurung[2], Sudipa Chakravarty[1], Amit Chakravarty[3]

1.Department of Genetics, Institute of Genetic Engineering . 30 Thakurhat Road. Kolkata- 700128, West Bengal, India.

2.Department of Genetics, Institute of Genetic Medicine and Genomic Science. 30A Thakurhat Road. Kolkata-700128, West Bengal, India.

3.Department of Biotechnology, Institute of Genetic Engineering. 30 Thakurhat Road. Kolkata- 700128, West Bengal, India

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Corresponding Author

Puspal De

Abstract

The aim of this study was to investigate the contribution of chromosomal anomalies and the frequency of particular types of aberrations in general couples preparing for pregnancy and make recommendations for pregnancy on the basis of the medical literature. A total of 84 general couples were included in the present study. The karyotypes were generated from the peripheral blood lymphocyte cultures and the cytogenetic analysis was performed using G-banding. In 84 couples, chromosomal anomalies were detected in 26 cases (30.95%, 26/84). Among them, the frequency of translocation was 4.76% (n=4), deletion was 5.95% (n=5) and autosomal derivatives was 1.19% (n=1). Sex chromosomal numerical anomalies accounted for 19.04% (n=16), including Klinefelter syndrome (KS) (n=3), Turner syndrome (TS) (n=3), and XYY syndrome (n=1). The others, including presence of mosaic cell lines was accounted for 10.71% (n=9).Our study indicates that clinically important chromosomal defects are present at a remarkable frequency in the general couples of eastern India, suggesting pre-pregnancy cytogenetic analysis should be routinely performed among general couples of this area, so that informed decision can be made, which will help to improve the quality of the pregnancy.

INTRODUCTION

Generally, the couples planning for their first pregnancy remain unaware of any reproductive problems. According to studies, approximately 1 in 6 couples experiences difficulties in reproductive outcome (O'Flynn *et.al*, 2010). It is estimated that the frequency of chromosomal aberration is approximately 8% in cases suffering from reproductive failure such as infertility and pregnancy loss (Du¨zcan *et.al*, 2003). About 15 – 20 % of all human pregnancies end in spontaneous abortion. Recurrent spontaneous abortion is historically defined as 3 or more constitutive pregnancy losses between 20 – 22 weeks of gestation period (Dubey *et. al*, 2005). However, some investigator feels that even 2 spontaneous losses constitutive and recurrent miscarriage deserves evaluation. In a patient with a history of two miscarriages, the subsequent risk of pregnancy loss rises to about 20 – 25 % whereas 3 abortions raise the risk of 4th miscarriages to 33 % (Middeldorp *et. al*, 2006). Another chromosomal abnormality, robertsonian translocation is recognized to be the most common structural abnormalities in the population with an incidence of 1.25/1000 live births (Gardner *et.al*, 1996). In Robertsonian translocation the pericentric region of acrocentric chromosome fuses to

form a single centromere or two. The resulting karyotype has only 45 chromosomes including the translocated one which is a result of long arm of two acrocentric chromosomes (Gardner *et.al*, 1996).

Approximately, 30 – 50 % of the case of male infertility is due to unknown reasons. In infertile couples half of the causes are male related, associated with impair spermatogenesis. Among the variety of reasons for male infertility, genetic factor is about 30 % of infertile male including chromosomal abnormalities and gene mutations (Quallich, 2006). It could be approximated that the overall incidences of chromosomal factors range between 2-8 % with a mean value of 5 % (Foresta *et.al*, 2002). This value increases about 15% in azoospermic males largely due to cases with 47,XXY aneuploidy. The most common type of abnormality observed in infertility is represented by Klinefilter Syndrome and also Y- chromosomal long arm microdeletion (Yoshida et.al, 1996). The Y chromosome deletion may be visible under microscope and it may be submicroscopic. If the deletion is at submicroscopic level, then it is called Y chromosome microdeletion.

In the present study we have screened 84 infertile couples with history of recurrent spontaneous abortions or missed abortions and infertility persist after 5-10 years of conjugal life. Age of the female partners range from 23 years to 41 years and the male partners' range from 25 years to 48 years. The incidence of spontaneous abortions occurred 3 to 8 in numbers. The whole abdomen USG reports of the female partners and the sperm count of the male partners and the hormonal profile of the both partners were also screened. The USG report of three female partners' shows different internal anatomical abnormalities like streak and apparently smaller sized ovaries, and hypostatic and rudimentary uterus. In a single case we observed azoopermia and in five cases we found moderate to severe oligospermia in male partners. Cytogenetic evaluation revealed presence of chromosomal abnormality in 26 cases (30.95%), among them 16 (19.04%) were female individuals and 10 (11.90%) were male individuals and both numerical and structural chromosomal abnormality observed. None of the patients were exposed to gonadotoxin such as radiation treatment and cancer chemotherapy. So, the present survey provides additional information to the growing literature in chromosomal abnormality related to infertility database.

Materials and Method

Suspected patients were examined by the clinician and then the characteristics of the patients were noted down. Biochemical profile and ultrasonography reports were included. Then patients' consent was taken and Cytogenetic analysis was carried out based on phytohaemaglutinin-stimulated peripheral blood lymphocyte culture method (Moorhead *et.al*, 1960) of all the couples. Lymphocyte culturing and GTG banding (Seabright, 1971) were performed following standard protocols as described by the AGT Cytogenetic Laboratory Manual. Karyotypes were described according to the International System for Cytogenetic Nomenclature (ISCN-2005).

The FISH was used to determine the presence or absence of gene at Xp21.2. and Yq11.3. Analysis was performed using DAX1 (NROB1) probe (BAC RP11-112945). (Red Signal) and double signals were visible from the centromeric X chromosome probe DXZ2 (Green Signals)(11) and LSI probe for Yq11.3 (Spectrum Green).

Results

Chromosome analysis of clinically diagnosed infertility cases revealed variant karyotypes like 45;X (3cases), 45;X/46;XX (5 cases), 45;X/46;XY (single case), 46XX/46XY (single case), and structural chromosomal abnormality found in the following 46;X;del(Xq) (2cases), 46;X;del(Xp) (single case), 45;XX(t14q:21q) (Single case), 46XX;t(5q35;8q24) (Single case) and 46XX;t(4,13)(single case) and 46,XX in female partners [Figure-1, Table-1] and 47;XXY(2 cases), 47;XYY(Single case), 45;X/46;XY(single case), 46;XY/47;XXY(single case), 46;XX/46;XY(Single case) and structural chromosomal abnormalities like 46;XY(delYq)(2 cases), 46;XY(der9q) (single case) and 46;XY,t(6q:8p)(Single case) in male partners[Figure-2, Table-2].

FISH analysis confirmed the gene deletion at Xp21.2. A signal was visible on only one X chromosome (Red Signal) at Xp21.2 and double signals were visible from the centromeric X chromosome probe (Green Signals). Another FISH analysis for SRY gene microdeletion on Y chromosome, Yq11.3 was also confirmed.

Table-1: Showing Different Chromosomal Abnormalities Observed In Female Individuals

Sl No	Type of Chromosomal Abnormality	Karyotype	No of Affected Female	% of Abnormality
1	Numerical	45;X	3	7.14%
2	Numerical	45;X/46;XX	5	11.9%
3	Numerical	45;X/46;XY	1	2.38%
4	Numerical	46;XX/46;XY	1	2.38%

5	Structural	46;X,del(Xq)	2	4.76%
6	Structural	46;X,del(Xp)	1	2.38%
7	Structural	45;XX,t(14q:21q)	1	2.38%
8	Structural	46;XX,t(5q35:8q24)	1	2.38%
9	Structural	46;XX,t(4q:13q)	1	2.38%

Table-2: Showing Different Chromosomal Abnormalities Observed In Male Individuals

Sl No	Type of Chromosomal Abnormality	Karyotype	No of Affected Male	% of Abnormality
1	Numerical	47;XXY	2	4.76%
2	Numerical	47;XYY	1	2.38%
3	Numerical	46;XX/46;XY	1	2.38%
4	Numerical	45;X/46;XY	1	2.38%
5	Numerical	46;XY/47;XXY	1	2.38%
6	Structural	46;XY,del(Yq)	2	4.76%
7	Structural	46;XY,der(9q)	1	2.38%
8	Structural	46;XY,t(6q:8p)	1	2.38%

Figure-1: Chromosomal Abnormalities Observed In Female Infertility

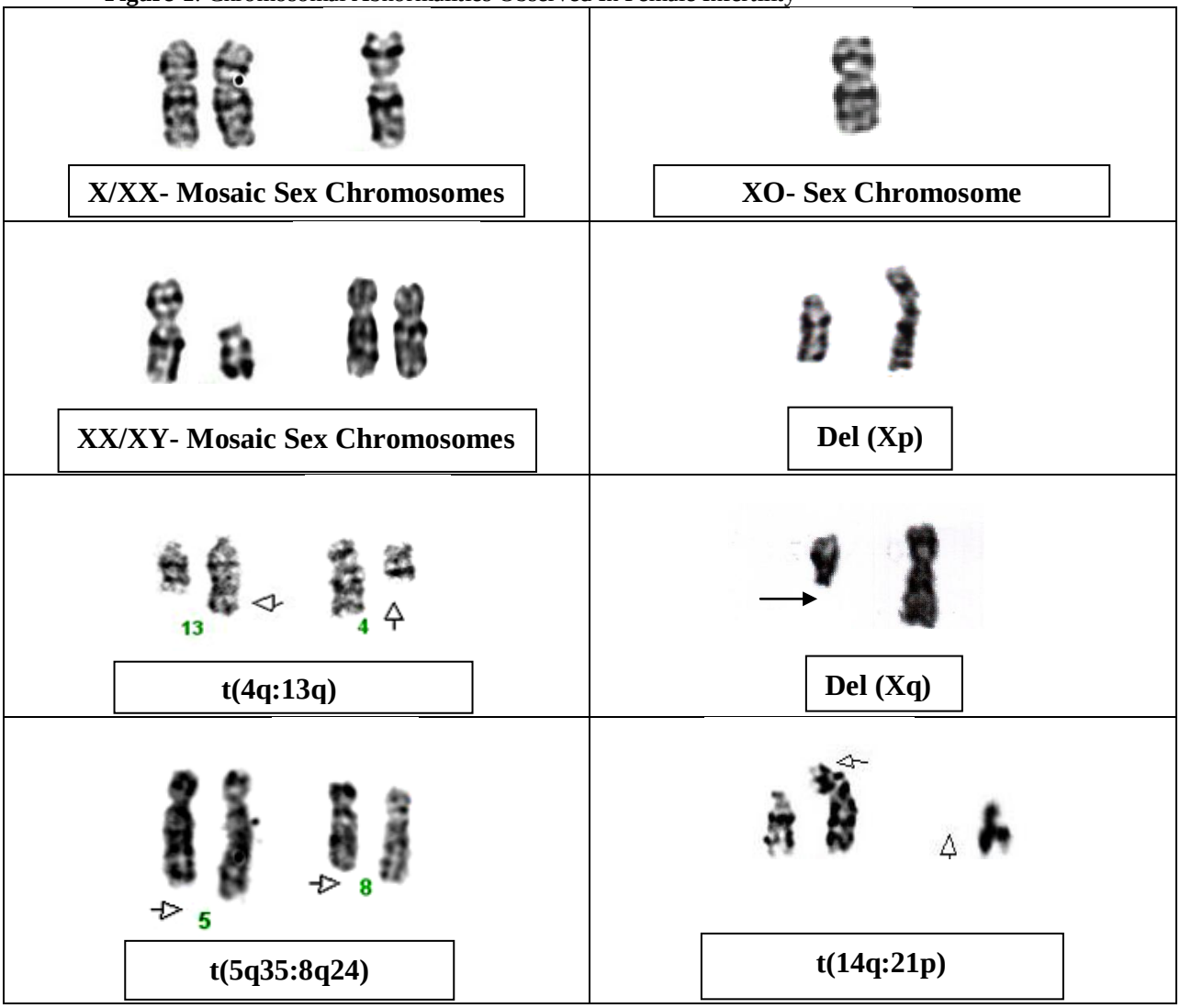
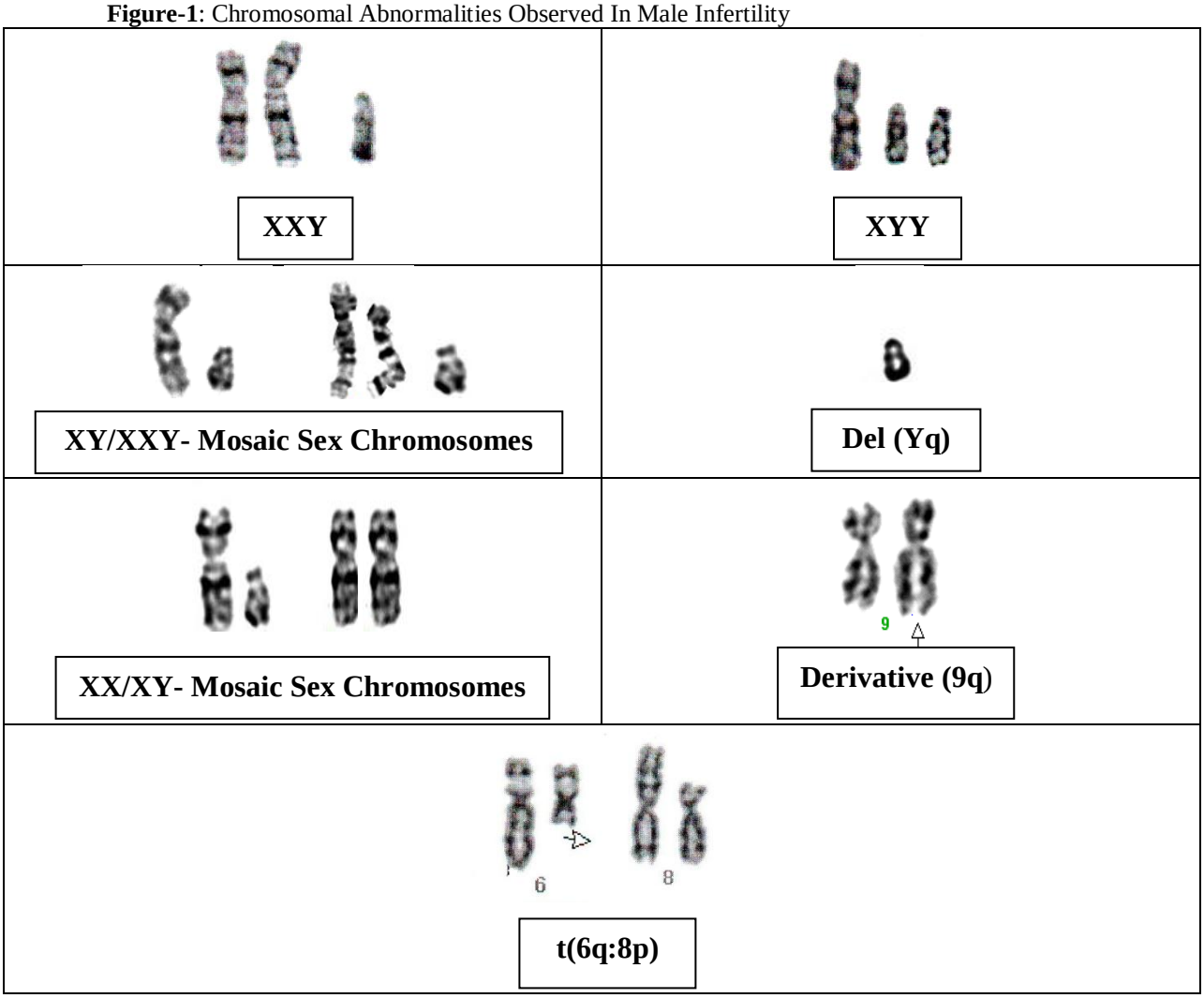


Figure-1: Chromosomal Abnormalities Observed In Male Infertility



Discussion

Humans have two types of chromosomes, autosomes and allosomes or sex chromosomes. In case of female individual, there are 22 pairs of autosomes and 1 pair of sex chromosomes while in male along with 22 pair's autosomes, single X and Single Y chromosome are present.

The evaluation of patients with a history of repeated spontaneous abortions requires careful consideration of potential genetic, anatomic, endocrine, infectious, and immunologic factors. Assigning proper etiological role to each of these contributing factors is often unclear, however specific information about the cytogenetic makeup of the couples and if possible of the abortus, still remains a primary focus during evaluation of such cases (Dubey *et.al*, 2005).

It has been reported that, the most common cause of spontaneous abortion in the first trimester (approximately 50%) is chromosomal abnormalities. The majority of chromosomal anomalies (95%) are numerical, about 60% are trisomy's, 20% are X monosomy and the remainder are (15%) polyploidy especially triploidy. On the other hand, half of the structural abnormalities may be inherited from a parent carrying a balanced chromosomal translocation which is at a higher risk of having children with chromosomal abnormalities (Shaffer *et.al*, 2007, Tsui *et.al*, 1996, Ogasawara *et.al*, 2000 and Regan *et.al*, 2000). It has been reported in some related articles that, the risk of RSA is increased in couples where one of them has such balanced rearrangement of the normally fertilized embryo 20%

were abnormal segregation of the translocation. This is considerably higher than the theoretical risks at prenatal diagnosis, probably because in *in vivo* most abnormal embryos would fail to establish a pregnancy. Screening of the embryos with an unbalanced product of the robertsonian translocation prior to birth would be expected to increase the chance of a successful pregnancy.

The short arm of X chromosome (Xp) helps in somatic development. The SHOX (short stature homeobox) gene, located on the 'p' arm of the X chromosome (Xp22.33), which encodes a transcription factor for skeletal development (Jacobs *et.al*, 1997, Chen *et.al*, 2009). Thus, Patients with monosomy X or deletion or duplication in Xp causes skeletal abnormality like short stature (Therman and Susman, 1990, Kaiser *et.al*. 1977). In our present survey the two cases with del(Xq) have the normal stature. But deletion in the long arm of X chromosome produces several degree of gonadal dysgenesis which causes recurrent spontaneous abortion. In a single case we have found deletion in short arm of X chromosome which creates moderately short stature and gonadal dysgenesis. In this case, though the short arm of X chromosome was affected, the body stature is also affected along with gonadal dysgenesis.

Apart from the above mentioned different types of structural chromosomal abnormality, in our present study, chromosome analysis of clinically diagnosed infertility cases revealed several types of numerical cellular mosaicism. Along with the classical aneuploids like 45;X we also observed 45;X/46;XX, 45;X/46;XY and 46;XX/46;XY in female partners and 46;XX/46;XY, 46;XY/47;XXY mosaicism in male partners. Clinically we found most of the patients have apparently normal phenotypes but The USG of whole abdomen in case of females shows smaller ovaries and hypostatic uterus. So, the presence of abnormal cell lines causes internal abnormalities and presence of abnormal cell line in greater percentage increased the severity. But clinically we could not found any anatomical or phenotypical abnormalities in male individuals with mosaic cell line. In two cases we have found infertility with normal stature and normal female karyotype 46;XX. Endocrinological disorder or abnormal hormonal regulation or pituitary dysfunction may cause spontaneous abortion or infertility in these cases though the hormonal profile was apparently normal. So, it will be the future area of interest.

Reciprocal or balanced (non-Robertsonian) translocations are one of the most frequently occurring human chromosomal aberrations (Dyke *et.al*. 1983). These rearrangements are twice more common in females than males. In most cases, carriers of balanced reciprocal translocations have a normal phenotype but may experience reproductive issues such as infertility or multiple miscarriages. 6 % of apparently balanced *de novo* translocations are associated with clinical abnormalities (Warburton. 1991). Recently, it has been shown by molecular analyses (e.g., array comparative genomic hybridization) that up to 40 % of the apparently balanced reciprocal chromosome translocations in patients with an abnormal phenotype are accompanied by a chromosomal imbalance (Sismani *et.al*. 2008). The present study revealed two unique balanced translocations in females such as 46;XX,t(5q35:8q24) and 46;XX,t(14q:21q) and a single balanced translocation in male partner 46;XY,t(6q:8p) with reproductive failure. Most of chromosomal abnormalities can be readily diagnosed with standard cytogenetic analysis. However, further refinements like subtle chromosomal rearrangements and intrachromosome exchanges can be identified by advanced molecular cytogentic techniques such as High Resolution Banding analysis or chromosome profiling. Couples with balanced reciprocal translocation have a 50 % chance of having recurrent spontaneous abortions and a 20 % risk of having children with abnormal genetic makeup (Wirth *et.al*. 1996). The formation of balanced, unbalanced and normal gametes is dependent on the basis of the breakpoints and also on the chromosomes involved. Balanced chromosomal translocations may also lead to sequence rearrangements of the functional genes which may result in the reproductive errors accompanied by repeated abortions (Farcas *et.al.* 2007). Further break point analysis and molecular characterization involved here might enlighten to understand the basis of recurrent spontaneous abortions.

A Robertsonian translocation is an unusual type of chromosomal rearrangement caused by two particular chromosomes joining together. When the translocation is balanced, the person with it, is called a Robertsonian translocation carrier. As carriers are healthy and have a normal lifespan, it is difficult to adjudge their unusual chromosomal rearrangement. In fact, the translocation can be passed down in families for many generations without anyone discovering. An unbalanced Robertsonian translocation may come to light only after a baby is born with a chromosome disorder. Most babies with unbalanced Robertsonian translocations have parents with normal chromosomes. Only a minor percentage of babies have one parent as a Robertsonian translocation carrier. In a Robertsonian translocation, two of the five acrocentric chromosomes present in the Group D (Tsui *et.al*, 1996, Ogasawara *et.al* and 2000, Regan *et.al*, 2000) and Group G (Warburton. 1991 and Sismani *et.al*. 2008) have broken at the beginning of the short arm near the point of centromere, where it meets the long arm. The long arms have then fused together, thus it is called centric fusion. This chromosome then consists of two long arms but no short arms. A

Robertsonian translocation carrier will have no major health problems due to their chromosome rearrangement and thus can remain unnoticed. Robertsonian translocation carriers do have an increased risk of pregnancy loss. While a few babies with trisomy 13 or 21 will survive, those with trisomy 14, 15 or 22 usually miscarry in the first twelve weeks. It has been suggested that certain robertsonian translocation carriers are particularly prone to pregnancy loss (Scriven *et.al*. 2001). In the present study, Cytogenetic evaluation revealed robertsonian translocation carrier in female partner with karyotype of 45,XX,rob(14q.21q). The couple has the history of three repeated abortions without any chromosome analysis report of aborted fetuses, we could not have any confirmatory idea about these repeated abortion. We suppose to think that the translocation derivative chromosome during segregation may cause non viable pregnancy in this case.

Chromosomal abnormalities are one of the most important cause of male infertility. The incidence of cytogenetic abnormalities has been estimated to be 2.1–28.4% in infertile men and only 0.7–1% in the general male population (Vutyavanich *et.al*. 2007). Chromosomal anomalies in the infertile male may be numerical or structural and involve sex chromosomes (e.g., 47,XXY) or autosomes (e.g., balanced Robertsonian translocations) (Vutyavanich *et.al*. 2007). Approximately 5–10% of the oligozoospermic and 15–20% of the azoospermic cases harbor genetic abnormalities (Dada *et.al*. 2006). The main genetic factors involved in male infertility are chromosomal abnormalities and Y-chromosomal deletions within the Yq11 region. The genes controlling spermatogenesis located in the Yq11 region are termed azoospermia factor genes (AZF) (Vogt *et.al*. 1992). According to the literature, among the AZF-genes, AZFc is the most frequently deleted one (60%), followed by deletions of AZFb and the combined deletions involving different AZF regions (35%), whereas AZFa deletions are extremely rare (5%) (Pina-Neto *et.al.* 2006). AZF subregions act in different phases of spermatogenesis. The complete deletion of the AZFa region is suggested to result in complete Sertoli cell-only syndrome and azoospermia (Simoni *et.al*. 2004 and Vogt *et.al*. 1996). Deletions of the AZFb region may induce SCO syndrome or the arrest of spermatogenesis in the primary spermatocyte stage (Yang *et.al*. 2008). Deletions in the AZFc region produce a variety of phenotypes ranging from normal to oligozoospermia and azoospermia (Simoni *et.al*. 2004 and Zhang *et.al*. 2007). Deletions in the AZFd are likely to present with mild oligospermia or even normal sperm counts with abnormal sperm morphology, such as severe teratozoospermia phenotype (Ceylon *et.al*. 2009). A deletion of the AZFc region may also predispose men to Y chromosome loss, leading to sexual reversal. Several studies have found this deletion to be a premutation for 45,X (Zhang *et.al*. 2007 and Jaruzelska *et.al*. 2001) and for the mosaic phenotype 45,X/46,XY (Patsalis *et.al*. 2002). In the present study we found single case of 45,X/46,XY in male partner, but deletion in AZFc region could not be discovered. We also observed three cases with deletion in long arm of chromosome Y. out of three, two cases are diagnosed by routine karyotyping but in a single case we confirmed the deletion in Yq11.3 region by FISH method. All the above mentioned Y chromosome deletion cases have the history of moderate to severe oligosprmia. Chromosome analysis of Single azospermic and other two oligospermic males revealed 47;XXY chromosomal constituents. No sperm production or low sperm count may cause infertility in these cases.

Conclusion

The present study and literature review showed that infertility had a higher prevalence of chromosomal abnormalities, even though they did not show any phenotypical features of a particular genetic disease. Chromosomal abnormality affects 30.95% in infertility, among which 19.04% were female, related and 11.90% were male related. Therefore, our study reemphasized the need of chromosomal evaluation for a problem like infertility and evaluating couples who need assisted reproductive technologies for genetic counseling.

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Patients Consent Statement: The signed consent from all the patients were taken before test was performed and kept them as official documents. In case of any unusual condition it will be presented in front of the concerned person.

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