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Analysis of genetic abnormalities in male infertile patients

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Abstract— Background and aim: The inability of a man to make his clinically fertile woman partner pregnant is known as male infertility. Several published studies have demonstrated that men who are infertile have a greater risk of chromosomal abnormalities than those who are fertile. Male infertility has been linked to various variables, including genetic and environmental factors. This study aimed to examine the hormonal changes and establish the incidence and kind of chromosomal alterations in infertile men with azoospermia and oligozoospermia. Materials and Methods: The traditional study of GTG-banded metaphases from cultured lymphocytes was conducted on 120 individuals with primary infertility. The retrieved data included medical history, family history, sperm analysis, and hormone profiles. The polymerase chain reaction was used to detect Yq11 micro deletions in all men with oligospermia/azoospermia with normal karyotypes. Results: The incidence rate of chromosomal abnormalities was 5.8%. A total of 65.8% of the men exhibited azoospermia, with 6.3% having chromosomal abnormalities. Oligozoospermia affected 33.3% of men, with 2.5% having chromosomal abnormalities. In 5.8% of cases (07/120), chromosomal abnormalities were detected. Two of the seven men had the karyotype of Klinefelter's syndrome, which is the most frequent numerical sex chromosomal abnormality. One patient had mosaic chromosomal aberration 46,XY (88%)/45,X (12%), and another had 46,XX (SRYpositive) chromosomal makeup. Karyotypes 46,XY, t (7;14) (q11;q22) (0.83%), 46,XY t (3;13) (p21;p11) (0.83%), and 46,XY, inv (9) (p11-q13) (0.83%) were found in 2.5% of patients. Conclusions: Our findings emphasized the necessity of cytogenetic testing in infertile patients before initiating therapy. Infertile individuals should have genetic testing and counseling to determine the reason for their infertility and assess the reproductive risk of couples with genetic disorders that may be passed down to their children.

Keywords: Azoospermia; Cytogenetics; Klinefelter syndrome; Male infertility; Oligozoospermia; Translocations.

Introduction:

Male infertility is described as the failure of a sexually active, non-contraceptive couple to conceive within a year. It is estimated that 60–80 million couples worldwide suffer from infertility each year, with more than 15–20 million in India alone.[1] Infertility affects around 15% of couples for various reasons, with the male component accounting for more than half of the cases.[2] Genetic factors play a key role in male infertility, with a reported frequency of 2%–8% in infertile men and up to 19% in men with azoospermia.[3] Even if the linked genes are unclear, the genetic factors may be implicated in idiopathic male infertility, which accounts for 40% of cases.[3] Furthermore, male infertility is the major cause of infertility in 20%–70% of couples.[4] Detecting genetic abnormalities is critical to the success of assisted reproductive technologies.[5] Chromosomal abnormalities are more common in severe testicular dysfunction, such as azoospermia and severe oligozoospermia. Klinefelter

syndrome (KS) is initiated by a non-disjunction of the X chromosome through meiosis, which affects around 1 in 1000 men. The karyotype 47,XXY, is often linked with KS and may be seen in all cells or in mosaic form.[6] KS is the most frequent numerical genetic anomaly, with a frequency of 5% and 10% in men with severe oligozoospermia and azoospermia, respectively.[4] It is caused by an additional X chromosome, which causes severe testicular dysfunction and spermatogenesis failure.[7] Men with KS have hypogonadism, azoospermia, tiny testes, erectile dysfunction, and increased gonadotropin levels compared with fertile men.[8] The gonadal abnormality in XXY men appears to be linked with germ cell existence and sex chromosomal makeup. The spermatogenic profiles of XYY men vary greatly, ranging from severe damage to apparent normalcy.[9] Other varieties of KS mosaics and 47, XYY men were mostly affected by numerical sex chromosome alterations. Both men with azoospermia and those with oligozoospermia showed these alterations.[10] Patients with XX male syndrome are less prevalent than patients with KS. [9]. The irregular cross-over between the X and Y chromosomes can result in an extra X chromosome carrying the SRY gene through a translocation mechanism.[10, 11] The Y chromosome–specific SRY gene is one of the most important genes in sex determination. SRY is a 204-amino-acid protein that codes for a testis-specific transcription factor involved in sex determination and is located on the p-arm of the Y chromosome.[12] It is a short, intronless gene with a conserved DNA-binding high-mobility group box, indicating that it regulates gene expression.[13] Male external genitalia, micropenis, hypospadia, and cryptorchidism are all symptoms of the XX male condition.[9] Chromosome translocations, inversions, and Y chromosome microdeletions are examples of structural genetic disorders. Reduced fertility is caused by autosome–autosome translocation because the translocated chromosomes must synapse via a pairing cross to continue through meiosis.[14, 15] An inversion occurs when a single chromosome splits into two pieces and is reassembled, with the portion in the middle reversed. A carrier of any kind of inversion is associated with the risk of developing an aberrant gamete due to inversion. 46,XY, inv (9) (p11q13) in infertile men is the most well-known reversal or pericentric inversion.[16] Male infertility is caused by Y chromosome abnormalities. Essential genes on the q arm of the Y chromosome have long been recognized as necessary for proper spermatogenesis.[17] The AZF gene is one of the most studied Y chromosomal genes in infertility research. Through homologous recombination, the AZF chromosomal region comprises repetitive homologous sequences that are prone to deletion, duplication, or translocation.[18] Whole AZF gene deletions are uncommon in the general population, but roughly 10% of individuals with idiopathic infertility have complete or partial AZF gene deletions.[19] As a result, this study aimed to find out the prevalence of chromosomal and gene changes in infertile men with primary infertility.

Materials and Methods

The control volunteers and patients gave their informed consent to participate in this study. Both the hospital and the university's ethics and bio safety committees provided their approval to the present study. A total of 120 male patients with infertility were sent to our lab for genetic testing. Seven of them had chromosomal abnormalities. The control group consisted of 75 viable men with normozoospermia and had fathered at least 1 child. Men who were sexually healthy and physically fit were included in the control group. They were

matched by age, caste (ethnicity), and geography to prevent mixed findings due to population stratification. The male patients had a regular diagnostic examination including a clinical examination, sperm analysis according to the World Health Organization (WHO) criteria, and a hormonal profile. [20]. The Male Infertility Clinic, Department of Family Welfare Centre, Lok Nayak Jai Prakash, and Associated Hospitals in New Delhi, India, were used to recruit infertile men. They were questioned, and the information regarding their medical history (mumps, diabetes, kidney disorders, endocrine abnormalities, exposure to environmental contaminants, and drugs) impacting spermatogenesis was gathered. Patients with any of the aforementioned symptoms, as well as heavy alcohol use, smoking, or radiation exposure as part of radiotherapy, were excluded from the study. The patients were assorted into two groups based on the semen analysis and WHO criteria: nonobstructive azoospermic and severe oligozoospermic.

Cytogenetic analysis: All patients were cytogenetically examined using standard procedures on phytohemagglutinin-stimulated peripheral-blood culture. The peripheralblood samples were used for harvesting according to the standard procedure.[21] Trypsin and Giemsa stain were used to stain G band chromosomes[22] The anomalies in G-banded metaphase spreads were reported using the International System for Human Cytogenetic Nomenclature 1995.[23]

Molecular analysis: Proteinase K digestion, phenol-chloroform isolation, and isopropanol precipitation with cold 3M sodium acetate (pH 5.2) were used to separate the genomic DNA from the peripheral-blood leukocytes of the patients and normal male controls. After vacuum-drying the DNA samples and dissolving them in Tris-EDTA, the quality and amount of genomic DNA were determined using the gel electrophoresis method.

Polymerase chain reaction analysis: The polymerase chain reaction (PCR) amplification was used to investigate the alterations in the SRY and the AZF areas on the Y chromosome. The primers for PCR were used to diagnose the micro deletion and mutations in these areas. The overlapping primers were employed to test the SRY gene. In PCR, two sets of primers were used to magnify the parts of the 254 and 351-bp areas of the SRY gene. In addition, as a control, one pair of primers was used to amplify the exon 5 of the p53 gene. In these 120 infertile men, multiplex PCR (M-PCR) was used to detect AZF-specific STSs on genomic DNA. Multiplex PCR was used to amplify the genomic DNA of patients with Y chromosome sequence-tagged sites.[24]PCR amplification was performed using Taq DNA polymerase in a 25-µL mixture containing 10 mmol/L Tris-HCl (pH 8.4), 50 mmol/L KCl, 1.5 mmol/L MgCl2, 200 mol/L each of deoxynucleotide triphosphates, 5 pmol primers, and 100–500 ng patient's DNA. The following PCR cycle parameters were used: a 4-min denaturation step at 95°C, 30 s at 55°C, 30 s at 72°C, and a 5-min extension phase at 72°C. Each PCR comprised both negative and positive controls. Ethidium bromide was used to conduct PCR on 2% agarose gels using an ultraviolet transilluminator. A 100-bp ladder was used as a size reference on all agarose gels.

Results: This study focused on the incidence and kinds of chromosomal abnormalities found in 120 men who had primary infertility and healthy spouses. The chromosomal study of all

female partners confirmed that they had a normal karyotype. The sperm examination demonstrated 79 men with azoospermia (65.8%), 40 men with oligospermia (33.3%), and 1 man with asthenozoospermia (0.8%). Further, the control group demonstrated 75 men with normozoospermia. Also, 113 (94.2%) of the 120 infertile men had a normal 46,XY chromosomal composition, whereas the remaining 7 (5.8%) exhibited genetic aberrations. Infertile men with azoospermia and oligospermia had higher FSH and LH levels, but testosterone levels were typically lower. Normal levels of testosterone, LH, and FSH were found in men with normozoospermia. Table 1 shows the numerical and structural chromosomal abnormalities found in 07 individuals. Chromosomal abnormalities were found in 5 (6.3%) of 79 men with azoospermia and 1 (3.2%) of 31 men with oligozoospermia. In four (3.3%) men, numerical sex chromosomal anomalies were detected. The 47,XXY karyotype (KS) was detected in two men (1.6%) and was the most prevalent numerical aberration. One man with azoospermia had a mosaic chromosomal composition of 46,XY (88%)/45,X (12%). One (0.8%) of the infertile men had the 46,XX chromosomal makeup. The SRY gene was found to be intact; however, the AZF region was removed. This resulted in (SRY+) 46,XX male sex reversal. In three instances (2.5%), structural chromosomal abnormalities were discovered. Translocations were shown as 46,XY t(3;13) (p21;p11) and 46,XY,t(7;14) (q11;q22) in men with oligozoospermia and asthenozoospermia, respectively. Pericentric inversion was discovered as 46,XY, inv (9) (p11;q13) in one infertile man. In terms of micro deletions, Y chromosome micro deletions were found in five (4.2%) infertile men. Out of 120 men with infertility, AZFc micro deletions were found in 3 (2.5%) men with azoospermia and 2 (1.7%) men with oligozoospermia. They were all infertile men with micro deletions in the AZFc area, which might be due to the huge size of the AZFc region. The fathers and brothers of the men excluded from the study were also evaluated, but no deletion was found in them. Also, the 46,XY chromosomal makeup was normal in all five cases. No chromosomal defects or Y chromosome micro deletions were found in any of the 113 infertile men. None of the men in the control group had any chromosomal or genetic abnormalities.

Discussions: The most serious human health issue throughout the reproductive years is infertility, which is defined as the inability to conceive after a year of unprotected sexual activity.[25] The most prevalent genetic abnormalities in infertile men are cytogenetic abnormalities, which have been suggested to be one of the primary factors contributing to male infertility.[26] In the present study, 5.8% of infertile men were found to have an aberrant chromosomal profile, which was comparable to previous investigations reporting chromosomal aberrations in infertile men ranging from 2.4% to 16.4%.[27] These findings highlighted the need for cytogenetic testing in infertile patients. Several forms of chromosomal abnormalities were discovered in this study. In the present study, 3.3% of the participants had sex chromosomal abnormalities. In some individuals with all metaphases analyzed, the loss of the largest section of the q arm of the chromosome (Yq11.2–qter) that might contain the region of spermatogenesis, which is the most common anomaly, was observed.[28] The most significant factor influencing the frequency of chromosomal alterations in men with infertility is the selection of men based on sperm count. The prevalence of chromosomal anomalies in men with azoospermia was 05/79 (6.3%) in the

present study, which was quite low compared with the findings in previous studies, 15.4%[29] and 4.6%.[30] The discrepancy in the incidence of chromosomal alterations identified in this study might be attributed to the ethnicity of the participants, the selection criteria, and the sample size.[31] Even in small samples of patients, several studies observed a significant proportion of sex chromosomal abnormalities. They found that infertile men with severe oligospermia and azoospermia had more chromosomal abnormalities. The most common genetic condition that causes infertility is KS.[32] It affects around 1 in 1000 men and is caused by a non-disjunction of the X chromosome during meiosis.[33] The chromosomal composition 47,XXY, which may be found in all cells or in mosaic form, is often linked to this condition. Men are typically infertile despite varying degrees of spermatogenetic failure.[34] We found two patients with Klinefelter karyotype (47,XXY), which is the most prevalent chromosomal defect that causes azoospermia in men. KS affects up to 5% of infertile men with severe oligozoospermia and 10% of infertile men with azoospermia.[35-37] The disorder has been associated with a higher number of X chromosomes (48,XXXY, 48, XYY, or even 49,XXXXY), as well as structural abnormalities in sex chromosomes.[38] Compared with normal and fertile men, men with KS have hypogonadism, azoospermia, tiny testes, erectile dysfunction, and increased gonadotropin levels.[39] The gonadal abnormality in XXY men is linked to the germ cell existence and sex chromosomal makeup. The failure of germ cells causes the testicular atrophy observed in patients with KS.[40] The most common cytogenetic numerical sex chromosomal defect seen in infertile men is KS, which is followed by translocations, deletions, and inversions.[40] This anomaly is linked to severe spermatogenic failure, which results in a significant decrease in testicular size as well as azoospermia, leading to infertility.[41] Sex reversal syndromes are associated with 46 XY women and 46 XX men.[40] 46 XX men is an uncommon disorder. It occurs in 1 out of every 20,000–25,000 births.[42] It has a 46,XX karyotype and is characterized by a masculine phenotype. The 46,XX male syndrome is divided into two categories based on the presence of the SRY region on the X chromosome: (SRY+) (80%) and (SRY–) (20%). Genital malformations and the loss of masculinization are usually identified in early infancy. Hence, men with the (SRY–) 46,XX male condition have a high rate of genital disorder and loss of masculinization.[43] Men with the (SRY+) 46,XX male condition transition through puberty normally; however, some develop cryptorchidism.[43] In early adulthood, these men are often identified with the aforementioned condition when undergoing tests for infertility. They have classic secondary sexual traits, yet they lack sperm. The testosterone levels may range from low to normal to high, and elevated gonadotropin levels are often seen.[43] We found one case of individuals with the (SRY+) 46,XX male condition in this study. An additional X chromosome harboring the SRY gene might develop from an irregular cross-over between the X and Y chromosomes through a translocation mechanism.[42, 43] Male external genitalia, micropenis, hypospadia, and cryptorchidism are all symptoms of the XX male condition.[41] In infertile men, autosomal translocations are the most prevalent structural chromosomal defects. The autosomal translocations suppress the spermatogenesis process due to altered meiotic pairing and segregation[44, 45] Infertile men have 4–10 times the number of autosomal translocations as fertile men.[46] Although most translocations have little effect on other organs, they may have a significant impact on spermatogenesis. We discovered translocations in two infertile men and a pericentric

inversion in one infertile patient. In our study, the rate of chromosomal alterations was lower than that reported in published studies.[47] Reduced fertility is caused by autosome–autosome translocation because the translocated chromosomes must synapse via a cross-over to continue through meiosis.[48] Robertsonian translocation is the most prevalent structural chromosomal anomaly among sterile men.[49] This is ascribed to the spermatogenesis-related gene disruption or defective synaptic complex pairing during meiosis. The transcriptional silencing of unpaired regions during meiosis in reciprocal translocation carriers has been postulated in the literature.[50] In the present study, structural chromosomal abnormalities were found in three patients (2.5%). Translocations in 46,XY t(3;13) and (p21;p11), 46,XY,t(7;14) (q11;q22) were discovered in men with oligozoospermia and asthenozoospermia, respectively. In one case, pericentric inversion was identified as 46,XY, inv (9) (p11;q13).[51] Inversion occurs when a single chromosome splits into two pieces and is reassembled, with the portion in the middle reversed. A carrier of any kind of inversion runs the danger of developing an aberrant gamete due to inversion. In sterile men, the most well-known reversal is 46,XY, inv (9) (p11q13).[52] Chromosome 9 inversion was identified in our study. The most prevalent human chromosomal inversions are pericentric inversions on chromosome 9, which result in asymmetric bivalents in meiotic metaphase I spread and structural chromosome abnormalities in sperm investigations. This inversion is believed to occur 1%–1.65% of the time in the general population. Although chromosome 9 inversions have no phenotypic impact, they have been linked to male infertility.[53]. Infertile patients with chromosomal abnormalities had much greater FSH and LH levels than those with idiopathic infertility, whereas men with chromosomal anomalies had lower blood testosterone levels. We also found that men with KS had higher levels of FSH and LH, as well as translocation. The male-specific region of the Y chromosome family of genes is found in the non-homologous region of the Y chromosome. They regulate the development of gonads and spermatogenesis, and hence may have a role in male infertility. Several genes on the Y chromosome have been shown to alter spermatogenesis.[54] On chromosome Y, the zinc finger Y-related protein (ZFY) gene plays a role in spermatogenesis.[55] The microdeletion of the Y chromosome in the ZFY region may impact men's fertility. Male infertility is caused by Y chromosome abnormalities. Essential genes on the q arm of the Y chromosome have long been recognized as necessary for proper spermatogenesis.[56] The AZF gene is one of the most studied Y chromosomal genes in infertility research. Through homologous recombination, the AZF chromosomal region comprises repetitive homologous sequences that are prone to deletion, duplication, and translocation.[57] Complete AZF gene deletions are uncommon in the general population, but roughly 10% of individuals with idiopathic infertility have complete or partial AZF gene deletions.[58] The male offspring inherits the AZF deletion. The pseudoautosomal region genes of the Y chromosome demonstrate similarities between chromosomes X and Y.[59] In all 120 individuals with infertility, AZFc de novo microdeletions were found in three men with azoospermia (2.5%) and two men with oligozoospermia (1.7%) because their brothers and fathers were not identifiable. All deletions in this study were novel. The AZFc gene is longer than the AZFa and AZFb genes, and the deletion in the AZFc gene is more common. The phenotype of AZFc deletion is milder and results in a variety of phenotypes ranging from hypospermatogenesis to SCOS, as well as the danger of transferring a corrupt gene to the male progeny in certain situations. In 2% of male

infertility cases, microdeletions in these locations result in severe testiculopathy and infertility.[60] In selected men with severe oligozoospermia or nonobstructive azoospermia, the incidence of Yq microdeletions increases to 15%–20%.[61]The findings of this study provided further evidence that these genetic aberrations were risk factors for sperm quality and infertility. Genetic testing is highly recommended to obtain reliable genetic evidence because of the high prevalence of cytogenetic abnormalities and Yq microdeletions in patients. It may be useful prior to ART management because people with microdeletions have less likelihood of producing children without ART.

Conclusions: Chromosomal abnormalities and Y chromosome micro deletions affected 5.8% and 4.2% of infertile men in this study, respectively. In addition, sex chromosome abnormalities were found to be more common in patients. The hormonal state of individuals with chromosomal abnormalities is affected, with considerable increases in FSH and LH levels. This results in greater negative effects on spermatogenesis, as seen by the decreased sperm retrieval rate in patients with chromosomal abnormalities. Further investigation is needed to find any new genetic abnormalities and to have a better understanding of the reasons for male infertility. Searching for alternative genetic reasons may reveal a higher incidence of genetic disorders. Before starting an IVF/ICSI program, the chromosomal analysis and Yq micro deletion study should be undertaken, followed by genetic counselling if a genetic issue is discovered.

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Data Availability Statement: This paper contains most of the data that were generated and analyzed throughout this investigation. The corresponding author can be contacted for more information.

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Table 1: Chromosomal anomalies amongst patients with azoospermia and severe oligozoospermia.

Cytogenetic Study	Karyotype	Number of patients with Sperm Count	%	Number of patients
Normal Chromosomal constitution	46,XY	Azoospermia-74 Oligospermia-39	61.6 32.5	Elevated FSH, LH & reduced testosterone
Numerical Anomalies				
Klinefelter's syndrome	47,XXY	Azoospermia-02	1.7	Increased FSH, LH & decreased testosterone
	46,XY(88%)/45,X (12%)	Azoospermia-01	0.83	Elevated FSH, LH & Normal testosterone
	46,XX (SRY+)	Azoospermia-01	0.83	Low LH, testosterone & FSH high
Structural Anomalies				
	46,XY, inv (9) (p11;q13)	Azoospermia-01	0.83	Elevated FSH & LH
	46,XY, t(7;14) (q11;q22)	Asthenozoospermia-01	0.83	data not available
	46,XY t (3;13) (p21;p11)	Oligospermia-01	0.83	Increased FSH, LH & normal testosterone