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Study of Karyotyping and Y Chromosome Microdeletionscreening in Infertile Males with Azoospermia and Oligozoospermia Prior to Art

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ABSTRACT

Introduction: Infertility is defined as the failure to conceive after one year of unprotected sexual intercourse. About genetic causes, both chromosomal abnormalities identifiable by karyotype analysis and gene mutations or submicroscopic deletions of the Y chromosomal azoospermia factor (AZF) region can disrupt spermatogenesis. Recently, the techniques of testicular sperm extraction (TESE) and intracytoplasmic sperm injection (ICSI) have made it possible to assist men with azoospermia or severe oligozoospermia to attain successful fertilization and pregnancies.

Aims: To find out the frequency and type of abnormal karyotype and Y chromosome microdeletions in infertile males before ART.

Study Design: A retrospective randomized analytical study. In this study, we enrolled 100 infertile male patients willing to go for ART.

Results: In 100 infertile male patients, 72 were azoospermic and 28 were oligozoospermic. There was no significant difference in the mean ages of men with oligozoospermia (31.6) and azoospermia (33.1). The levels of FSH and LH in azoospermic males were significantly higher than those in the oligozoospermic males ($P = 0.001$). However, the levels of testosterone ($P = 0.749$) were not significantly different. Of 100 cases with male infertility, 89% had a normal karyotype (46, XY) and 11% had abnormal karyotype. Eight of the chromosomal abnormalities were gonosomal in patients with Klinefelter Syndrome and two were balanced reciprocal translocations involving autosomes. One of the translocations was 46,XY,t(3;7) (q24 ;q36) and the other was 46,XY,t(5;6) (q35 ;q21). All of those with Klinefelter Syndrome had azoospermia, but translocation carriers had oligospermia. 11.1% of azoospermic patients and 7.14% of oligozoospermic patients had chromosomal abnormalities. In the current study, Yq microdeletion was detected in only 1.38% of azoospermic cases, but not seen in other cases.

Conclusions: The results of this study showed that infertile men had a higher prevalence of chromosomal alterations. Therefore, the higher incidence of the chromosomal anomalies among the infertile males strongly suggested karyotyping and counseling before ART.

Key Words: Intracytoplasmic sperm injection, Techniques of testicular sperm extraction, chromosomal anomalies

Setting: 21st Century Hospital, Vapi, Gujarat, India

Duration of Study: January 2019 to June 2019

INTRODUCTION

Infertility is defined as the failure to conceive after one year of unprotected sexual intercourse. This problem affects approximately 10%–15% of couples worldwide, and male-related factors are accountable for half of this case. Several

factors are implicated in male infertility like hormonal abnormalities, male erectile dysfunction, infections, anti-sperm antibodies, exposure to chemical agents and radiations, testicular cancer, varicocele, genetic factors, and others. Thus, male infertility is a multifactorial syndrome encompassing a large variety of disorders. However, in about 30%–50% of

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male cases, the aetiology of infertility continues to be unknown.¹

About genetic causes, both chromosomal abnormalities identifiable by karyotype analysis and gene mutations or submicroscopic deletions of the Y chromosomal azoospermia factor (AZF) region can disrupt spermatogenesis, while CFTR mutations are related to obstructive azoospermia. Klinefelter syndrome (KS) is a chromosomal condition caused by the presence of an additional X chromosome. Various genotypes are related to this condition, the foremost common being 47, XXY in up to 80–90% of cases, while in approximately 10% of cases the genotype may be a mosaic form of 47, XXY/46, XY. Other rare variants are 48, XXXY; 48, XXYY; 49, XXXXY; 49, XXXYY; and 49, XXYYY and are related to more severe phenotypes.³ KS shows an estimated frequency of 1:600 within the general population, whereas in patients with non-obstructive azoospermia it's as high as 15%. In adults, the diagnosis is principally made due to infertility and/or sexual dysfunction. The reproductive phenotype of those individuals includes small, firm testes with hyalinization of seminiferous tubules with consequent spermatogenic failure. Over 90% of KS patients are azoospermic, and the remaining show crypto/severe oligozoospermia.²⁻⁴

Microdeletion of the azoospermia factor (AZF) region located on the long arm of the Y chromosome (Yq11) is considered the foremost common genetic cause of male infertility. The AZF region is split into three non-overlapping subregions called AZFa, AZFb, and AZFc, all of which are required for normal spermatogenesis. Microdeletions in these three regions are related to various spermatogenic alterations including Sertoli cell-only syndrome (SCOS), maturation arrest, and hypospermatogenesis. Specifically, the microdeletion of AZFa is relevant to complete SCOS and azoospermia. The absence of AZFb is related to maturation arrest at meiosis, whereas microdeletion of AZFc ends up in variable clinical and histologic phenotypes, starting from oligozoospermia to SCOS. Extensive studies have been carried on Y microdeletions in non-obstructive azoospermic and severely oligozoospermic patients, with a reported incidence ranging from 3% to 28. Therefore, disruption of AZF may be viewed as the commonest molecularly diagnosable reason behind spermatogenic failure in the setting of non-obstructive azoospermia or severe oligozoospermia.⁵

Recently, the techniques of testicular sperm extraction (TESE) and intracytoplasmic sperm injection (ICSI) have made it possible to assist men with azoospermia or severe oligozoospermia to attain successful fertilization and pregnancies. However, abnormal karyotype and Y microdeletions can be transmitted from infertile fathers to their male offspring, who could also experience infertility, through the procedure of ICSI.⁶ Thus, it's important to evaluate Y microdeletions and abnormal karyotype in male infertility before ART to provide appropriate

information to patients and prevent genetic defect to pass on to the subsequent generation.

AIMS

To find out the frequency and type of abnormal karyotype and Y chromosome microdeletions in infertile males before ART.

OBJECTIVES

- 1) To find out the frequency and type of abnormal karyotype in infertile males before ART.
- 2) To find out the frequency and type of Y chromosome microdeletions in infertile males before ART.

MATERIALS AND METHODS

- The present study was conducted on 100 infertile male patients who came for semen analysis in 21st Century Hospital, Vapi, Gujarat during the period from January 2019 to June 2019.
- This is a retrospective non-randomized analytical study including infertile male patients with azoospermia and oligospermia willing to go for ART.

Inclusion criteria

- Patients whose sperm counts were less than 15×10^6 /ml
- Patients having primary infertility.
- Patients having non-obstructive azoospermia.

Exclusion criteria

- Patients with obstructive azoospermia and patients with secondary infertility
- H/o medical disorders (e.g. diabetes, sickle cell disease, renal disease or liver disease)
- H/o endocrine abnormality (e.g. prolactinoma, hypogonadotropic hypogonadism)
- H/o exposure to toxins and/or medications affecting spermatogenesis
- H/o exposure to radiations
- Acquired and congenital structural defects of urogenital system (e.g. cystic fibrosis, Young syndrome)

Patient variable and data collection

- Patient's name, age, occupation and address were noted from the records.
- The duration of infertility for each patient was taken from the records.
- Serum concentrations of FSH, LH and testosterone of these patients were noted from the records.
- The results of karyotyping and Y chromosome microdeletion of these patients were noted from the records.
- As the study was a retrospective study, no ethical board approval was required.

OBSERVATIONS AND RESULTS

The present study was conducted in the Dept. of Medicine and Dept. of OBGY at Jawaharlal Nehru Medical College, Datta Meghe Institute of Medical Sciences. This study includes 100 infertile male patients, 72 were azoospermic and 28 were oligozoospermic.

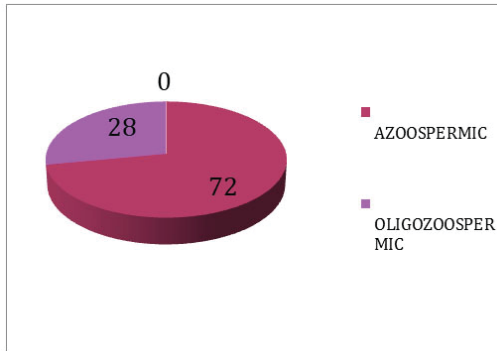


Figure 1: The categories of male patients taken in the study.

Table 1: The age distribution of the patients taken in the study

Age Distribution	No. of Patients
<25 Years	9
25-29 Years	27
30-34 Years	62
>35 Years	2
Total	100

Table 2: The duration of infertility in the patients taken in the study

Duration of Infertility	No. of Patients
1-5 Years	33
5-10 Years	48
>10 Years	19
Total	100

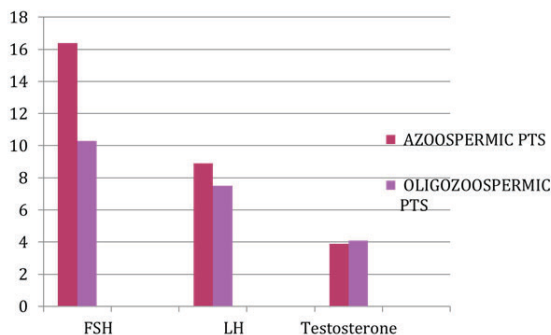


Figure 2: The average values of FSH and LH in IU/L and testosterone(ng/ml) in the patients taken in our study

Table 3: The results of karyotyping and Y chromosome microdeletion screening studies in the study:

	No. of Patients
Normal karyotype (46, XY)	89
Klinefelter syndrome (47,XXY)	8
Microdeletion in AZFc region	1
Microdeletion in AZFa and AZFb region	None
Balanced autosomal translocations	2

RESULTS

- In 100 infertile male patients, 72 were azoospermic and 28 were oligozoospermic (Figure1).
- The mean age of infertile males was 32.79 years. There was no significant difference in the mean ages of men with Oligozoospermia (31.6) and azoospermia (33.1)
- The average duration of infertility was 8.85 years (range 1-15 years) (Table no 1)
- The levels of FSH and LH in azoospermic males were significantly higher than those in the oligozoospermic males (P = 0.001). However, the levels of testosterone (P = 0.749) were not significantly different (Table 2 and Figure 2).
- Of 100 cases with male infertility, 89% had a normal karyotype (46, XY) and 11% had abnormal karyotype.
- Eight of the chromosomal abnormalities were gonosomal in patients with Klinefelter Syndrome and two were balanced reciprocal translocations involving autosomes. One of the translocations was 46, XY, t(3;7) (q24 ;q36) and the other was 46,XY,t(5;6) (q35 ;q21). (Table no 3)
- All of those with Klinefelter Syndrome had azoospermia, but translocation carriers had oligospermia.
- 11.1% of azoospermic patients and 7.14% of oligozoospermic patients had chromosomal abnormalities.
- In the current study, Yq microdeletion was detected in only 1.38% of azoospermic cases, but not seen in other cases.
- That patient presented the loss of sY277 and DAZ3 in AZFc region.
- Neither AZFa nor AZFb deletions were detected in any participant.
- The case with deletions had a normal karyotype (46, XY). Combination of chromosomal abnormality and Y chromosome microdeletion was not seen in any case.
- Including Y chromosome microdeletions and chromosomal abnormality, a total genetic abnormality rate was detected in 12.5% of azoospermic cases (9/72) (Table No 1) and 7.14% of oligospermia cases (2/28)

DISCUSSION

The occurrence of karyotypic abnormalities among infertile men depends on a variety of factors; the foremost important of those is that the criterion for selection of patients based on the sperm counts. The prevalence of chromosome abnormalities is higher in infertile men and it's well-known that the sperm count is inversely associated with the existence of chromosomal abnormality ⁵. The first study, emphasizing the relation between some chromosomal abnormalities and male infertility, included 6982 cases, and the frequency of chromosomal abnormality was reported as 5.3% ⁶⁺. The frequency in the whole population was given as 0.6%. within the same study, gonosomal and autosomal chromosomal abnormality ratios were found to be 15- and 6-fold, respectively.⁷⁻¹⁰ In our study among the 100 infertile men studied, 11 showed chromosomal abnormality corresponding to a frequency of 11%. The chromosomal abnormalities were more frequently observed in the population of azoospermic males than in the oligospermia males (12.5% vs 7.14%) similar to study conducted by Lissitsina et al. ⁸ In the present study, chromosomal abnormalities were detected in 12.5% patients of 72 azoospermic cases and 7.14% patients of 28 oligozoospermic cases. The chromosomal abnormality frequency in males with oligospermia was reported as 1.8–6.9% in several studies while in our study, a relatively high number (7.14%) of oligospermia cases had a chromosomal abnormality.¹¹⁻¹⁴

The most common form of karyotype abnormality in infertile cases is represented by Klinefelter syndrome (KS). Ferlin et al. reported that the prevalence of KS among infertile men is extremely high, up to 5% in severe oligozoospermia and 10% in azoospermia. ¹⁵ KS was the foremost frequent chromosome-related cause of infertility in our study group. In our study, we detected that 8% of cases had Klinefelter syndrome. All of the patients with the chromosomal abnormality of KS had azoospermia. Among azoospermia groups, they comprised 5.76%.

Among 11 cases who had a chromosomal abnormality, 2 cases had balanced reciprocal translocations involving autosomes. one of the translocations was 46,XY,t(3;7) (q24 ;q36) and the other was 46,XY,t(5;6) (q35 ;q21). Both of the translocations carriers had oligospermia. Among 28% of cases of oligospermia, only 0.56% had chromosomal abnormality which was balanced autosomal translocation. None of the oligospermia cases had Klinefelter's syndrome or Y chromosome microdeletion.

In the current study, only 1 patient had a Y chromosome microdeletion. Yq microdeletion was detected in barely 1.38% of azoospermic cases, but not seen in oligospermia cases. That patient presented the loss of sY277 and DAZ3 in AZFc region. Neither AZFa nor AZFb deletions were detected in any participant.

The case with deletions had a normal karyotype (46, XY). Combination of chromosomal abnormality and Y chromosome microdeletion wasn't seen in any case. Patients in azoospermia groups had higher FSH and LH levels than in oligospermia patients but levels of testosterone weren't significantly different. Serum FSH and LH levels and testicular volume correlate with the testicular function including sperm density, total sperm count. These findings are consistent with those of previous reports. Serum FSH, LH levels and testicular volume may have prognostic implications on testicular function, but we don't know whether these parameters have any prognostic implications on the cytogenetic abnormality of infertile patients. Our study has shown that patients with a chromosomal abnormality are vulnerable to have higher serum FSH and LH levels.

In conclusion, the results of this study showed that infertile men had a higher prevalence of chromosomal alterations. Therefore, the higher incidence of the chromosomal anomalies among the infertile males strongly suggested karyotyping and counselling before the employment of the assisted reproduction techniques. ¹⁵

CONCLUSION

In conclusion, the occurrence of chromosomal anomalies and Y chromosome microdeletions among infertile males strongly suggests genetic testing and counselling before employment of assisted reproduction techniques.

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