Original Research Paper

Volume-9 | Issue-8 | August - 2019 | PRINT ISSN No. 2249 - 555X

Anatomy

CHROMOSOMAL ABNORMALITIES DETECTED IN INFERTILE MALES – A RETROSPECTIVE STUDY

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ABSTRACT The present study was carried out to find out the frequency and types of chromosomal abnormalities in males attending the Infertility Clinic of Govt. Medical College, Kozhikode, which is a Tertiary Care Centre of North Kerala.

Study settings and design: A retrospective study of karyotypes of 319 infertile males was done. The results of cytogenetic analysis of male patients over a period of 5 years (2014 January to 2018 December) were studied.

Methods: Peripheral blood lymphocyte culture was done according to standard protocol and chromosomal analysis was carried out using Cytovision software version 7.

Results: The results of present study show that the incidence of chromosomal abnormalities in infertile men is 18.45% which includes structural and numerical anomalies. The remaining 81.55% males showed apparently normal karyotypes.

Conclusion: The results of this study indicate that in all phenotypic males with infertility, karyotyping should be done after excluding non-genetic causes. This will help in the precise diagnosis, management, possibility of ICSI, pre implantation diagnosis and genetic counseling for the couple.

KEYWORDS :

INTRODUCTION:

By the term "infertility", it is meant that the couple is unable to conceive. Infertility can be primary if conception has never occurred and secondary if the patient fails to conceive after having a previous conception. The incidence of infertility is approximately 5 -15% in any community.[1]

In one third of all cases of infertility, the male partner is directly responsible. The cause may be disorders of spermatogenesis, disorders of male reproductive system, disorders of sperm motility, hormonal imbalance, genetic causes and certain environmental factors.

The density of sperm population plays a major role in infertility. Absence of sperms is called azoospermia. Oligospermia is mild when the count is 10 -20million/ml, moderate when the count is 5-10million/ml and severe when sperm count is less than 5 million/ml.[1]

Oligo astheno terato zoospermia (OATS) is a condition in which sperm count is less, less than 50% of sperms in the semen exhibit forward motility and the proportion of sperms with normal shape is less than 30%.[2]

It has been shown over the past decade that genetic disorders form the basis of a large number of cases of male infertility[3,20] The development of Assisted Reproductive Technology (ART) has given hope for several couples suffering from infertility of the male partner.

The introduction of Intra Cytoplasmic Sperm Injection (ICSI) in early 1990s gave rise to solution for longstanding infertility to a certain extent. ICSI with sperm of poor quality gives better fertilization rates and yields more embryos capable of implantation in the uterus. This is why the chromosomal analysis in infertile male becomes mandatory.

More than 40 years age, it was first recognized that chromosomal anomalies are responsible for male infertility. In the infertile group, the sex chromosome anomalies are much greater than autosomal anomalies.

OBSERVATION AND RESULTS:

The distribution of chromosomal anomalies in males with azoospermia , oligospermia and OATS is shown in Table .1.

Table 1: Frequency of Chromosomal Anomalies – In the Present Study

Karyotype	Total Number of Cases (n=319)	Percentage
Normal	260	81.55
Abnormal	59	18.45

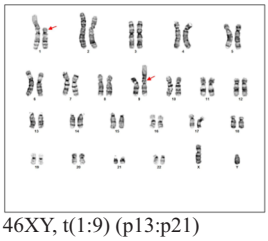
A total of 319 cases of males with azoospermia , oligospermia and OATS were evaluated for chromosomal abnormalities. 260 males (81.55%) had a normal karyotype (46XY), while 59 males (18.45%) had chromosomal anomalies which included numerical as well as structural anomalies. The details of karyotypes of males with infertility and frequencies of different chromosomal anomalies obtained in our study are shown in Table II.

Table : II - Chromosomal Abnormalities Detected in Infertile Men (n = 319)

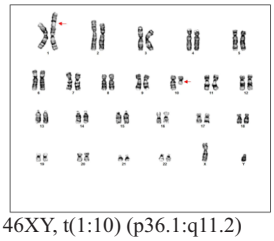
Abnormality	Total Number	Type of abnormality	No. of cases in each type	%
Numerical Abnormalities	31	Klinefelter's syndrome	30	9.4
		48 XXYY	1	0.31
Structural Anomalies	10	Translocations a) 46XY,t(11:13)(q21:q21.2) b) 46XY, t(13:15) (q34:q21) c) 46XY, t(1:9) (p13:p21) d) 46XY, t(1:10) (p36.1:q11.2) e) 46XY, t(2:3:12) (p.24:q29: q21.3) f) 46XY, t(7:14) (q34:q11) Derivatives: a) 46 XY der 1 b) 46 XY der 15 c) 46 X, der x Marker chromosome 47 XY + mar	6 3 1	3.13
Mosaic	1	Mos 46 XY (80%) / 45 X (20%)	1	0.31
Small Y	2	46 XY ?Microdeletion	2	0.62

Polymorphic variants	13	a) 46 XY 1 qh - (2 cases) c) 46 XY 15 ps + (2 cases) d) 46 XY 9 qh – (one case) e) 46 XY 22 ps+(one case) f) 46 XY, 21 pstk ++(one case)	13	4.07
Others	2	46 XX	2	0.62
Total	59		59 (out of 319 cases)	18.46%

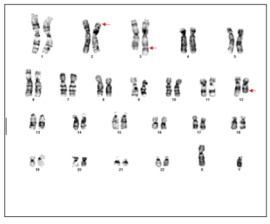
Different Types Of Chromosomal Anomalies Detected In Our Cytogenetic Lab:



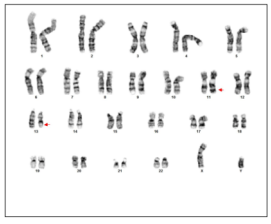
46XY, t(1:9) (p13:p21)



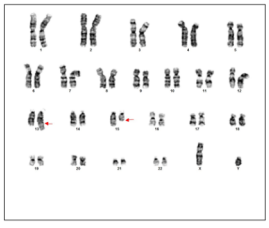
46XY, t(1:10) (p36.1:q11.2)



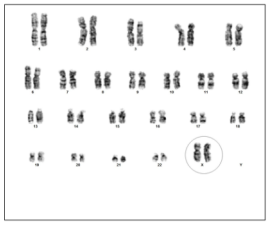
46XY,t(2:3:12)(p.24:q29:



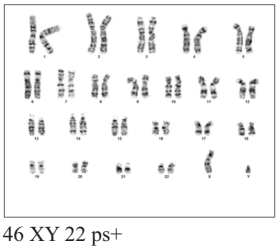
46XY,t(11:13)(q21:q21.2)



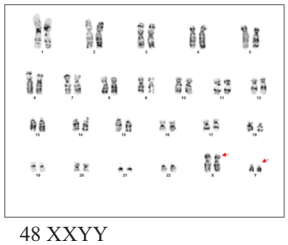
46XY, t(13:15) (q34:q21)



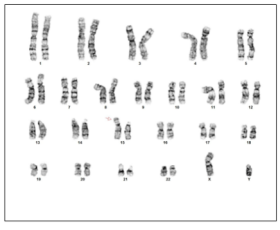
46XX (PHENOTYPIC MALE)



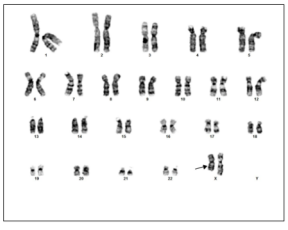
46 XY 22 ps+



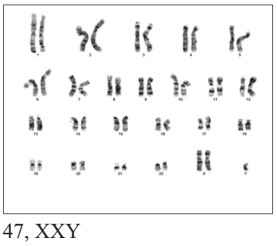
48 XXYY



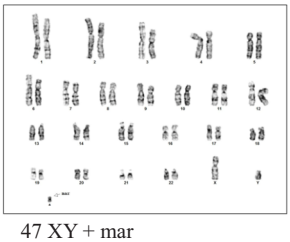
46 XY der 15



46 X, der x



47, XXY



47 XY + mar

DISCUSSION:

Indian families give lot of importance of having children of their own; a social stigma is attached to the couple who do not have a child.4 In the olden days, females were blamed entirely for not giving birth to a child. But, with the advent of various clinical tests, especially cytogenetic analysis, males were also found to be responsible in 40-50% of cases.5 Genetic studies conducted in different parts of the world have discovered that genetic anomalies are present in about 15% of infertile males, which include chromosomal aberrations and single gene mutations.6 The results of various studies are given in **Table III.**

Table III: Incidence of chromosomal anomalies in infertile males

Author	Year	Total No. of patients	Chromosomal anomaly (Number)	Percentage
Palka	1990	96	11	11.42
Gunduz	1998	102	16	15.7
Badovinac	2000	158	28	17.7
Alkalaf	2002	118	12	10.16
Lissitsina[7]	2002	27	05	18.5
Carp	2004	458	44	9.60
Quilter	2005	103	10	9.7
Sayee R	2006	73	12	16.4
Drugkar Amol	2007	70	09	12.85
PRESENT STUDY	**2018**	**319**	**59**	**18.46**

The results of present study is similar to that of Lissitsina (2002).7The chromosomal anomalies detected in infertile males are structural, numerical and mosaicism.[8]

Sex chromosomal abnormalities are reported to be more in male infertility4 Van Assche9 investigated 694 infertile males and found that 47XXY or Klinefelter syndrome predominate in male infertility.

Table IV shows the studies conducted by various authors and the incidence of klinefelter (KF) syndrome (47XXY) in each study.

Table IV : Incidence of Klinefelter Syndrome in Male Infertility

Author	Year	Total No. of patients	Klinfelter Syndrome	Percentage
Ismail[13]	1993	100	0	-
Yoshida[14,24]	1997	1007	28	2.78
Lissitsina [7,15]	2002	27	1	3.7
Ambasudhan[16]	2003	180	6	3.33
Abdelmoula[10]	2004	51	6	11.76
Sayee Rajangam[11]	2006	73	8	10.9
Quilter[17]	2005	103	2	1.94
Drugkar Amol[18]	2013	70	2	2.84
PRESENT STUDY	**2018**	**319**	**30**	**9.4**

Abdelmoula NB, Amouri A, Portnoi MF et al[10] have detected 11.4% males with Klinefelter syndrome. Sayee Rajangam[11], Tilak P, Aurana N, Devi R (2007) reported 10.95% patients with 47 XXY. The frequency of Klinefelter syndrome in our lab is 9.4%, which is closest to the values reported by Sayee Rajangam et al.

Translocations:

In infertile men, translocations are reported in 1.2% cases. Translocations may be Robertsonian (0.7%) or reciprocal (0.5%). Robertsonian translocations are usually observed in oligospermic males. Reciprocal translocations are associated with azoospermia (0.9%) and oligospermia (0.8%).[4]

Robertsonian translocations are the most frequent structural chromosomal anomaly found in infertile men. Van Asschi[9] and colleagues have reported 0.7% of the infertile males with this anomaly.

The impairment of sperm production by infertile carriers is explained by Johannison and colleagues[12,19]. According to these authors, a correlation exists between the increased frequency of the XY bivalent and the Robertsonian trivalent association, present during the pachytene stage of meiosis and the extent of germ cell impairment.[3]

Reciprocal Translocations:
There is a strong correlation between impairment of sperm production and reciprocal translocations with involvement of chromosomes 3,4,5,6,7,9,11,13,14,15,16,17,19,20,21 and 22.[21] This may be due to a structural effect related to alterations in the process of chromosome synapses during meiosis. Breakpoints on chromosome 1 were known to be associated with azoospermia and infertility.[22]

The incidence of translocations obtained by various authors is shown in **Table V.**

Table V: Incidence of Translocation in Male Infertility:

Author	Year	No.of cases	Translocations	Percentage
Baschat[23]	1996	32	02	6.25
Yoshida[24]	1997	1007	18	1.78
Haidl[25]	2000	305	10	3.27
Carp[26]	2004	458	21	4.58
Quilter[27]	2005	103	02	1.94
Sayee R[11]	2006	73	02	2.73
Drugkar Amol[18]	2007	70	01	1.42
PRESENT STUDY	**2018**	**319**	**6**	**1.86**

From this table, it is evident that the incidence of translocation in our centre correlates with the findings of Yoshida, Quilter and Drugkar Amol et al.

48 XXYY Syndrome:
Out of 319 cases of males with infertility, 1 patient showed this karyotype (0.31%). His phenotype was that of a normal male. Testicles were small in size, which was recorded in the Infertility Clinic Outpatient card. Patient also had azoospermia.

48 XXYY syndrome[23] causes infertility, developmental, behavioral disorders and other health problems. Adolescents and adult males with this syndrome have small testes which do not produce enough testosterone. This can lead to reduced facial and body hair, poor muscular development and gynaecomastia. These patients are usually tall , they are likely to develop tremor, peripheral vascular disease, deep vein thrombosis, pes planus , type 2 diabetes mellitus and congenital heart disease.

Cause : This syndrome affects one male in every 18000 - 40000 live births.[24] 48 XXYY syndrome results from the presence of an extra copy of both sex chromosomes in each somatic cell. Extra copies of genes on the X chromosomes interfere with male sexual development. On both X and Y chromosomes, there are genes in areas known as pseudoautosomal regions which contribute to the signs and symptoms of 48 XXYY syndrome.

The main cause of XXYY syndrome is an error in cell division called nondisjunction. In 48XXYY syndrome, the extra sex chromosome almost always comes from a sperm. Nondisjunction may cause a sperm to gain two extra sex chromosomes resulting in a spermatozoon with three sex chromosomes (one X and two Y), when this sperm is fertilized with a normal oocyte with one X chromosome, the resulting zygote will have 48 XXYY complement.

Nondisjunction can also occur, in a very small percentage, in a 46XY zygote, soon after fertilization.[25]

This syndrome was first reported in a boy by Sylfest Muldal and Charles H Ockey in Manchester, England in 1960.[26]

48 XXYY only affects males because at least one of the Y chromosomes with a properly functioning SRY gene will give rise to a phenotypically male individual.

46 XX Male:
There were two phenotypic males, presented with azoospermia and infertility. Their karyotype was 46XX.

The 46 XX maleness is characterized by testicular development, even though there is a lack of Y chromosome. The frequency of 46 XX males in general population is 1 in 10,000.

Three theories are put forward to explain the XX maleness.[4]
(1) Translocation of SRY gene (Sex determining Region of Y) from Y chromosome to the distal part of p-arm during meiosis (Van Der Auwera, 1992).
(2) Mutation in an autosomal or X-chromosome gene which permits testes development in the absence of SRY (Ferguson Smith, 1990).
(3) Undetected 46 XX / 47 XXY mosaics or other cells with Y chromosome.

In about 80% of 46 XX males, sex reversal is due to the presence of the SRY gene on one of the X chromosomes. This unusual localization of the SRY gene is due to the recombination during male meiosis between X and Y chromosomes near the pseudoautosomal region. The male has small testes and will be infertile

Polymorphic Variants:
In 13 cases out of 319(4.07%), polymorphic variants were detected. There were 6 cases of heteromorphism qh + fragile sites. Pseudosatellites were detected in 3 cases (46 XY 15 pst and 2 cases of 46 XY 22 pst). Dana Mierla[28], Dumitru Jardan et al report 9.06% polymorphic variants in their study. They have also reported that the polymorphic variants are more frequent in normospermic men. It was 1.6 times more frequent than azoospermic males.

Small Y Chromosomes (? Microdeletion)
In 2 cases (0.62%) Y chromosomes were found to be small. This may be due to microdeletion of Y chromosome (At the time of study, FISH and PCR were not available in our lab).

Genes necessary for spermatogenesis are located in the Y chromosome. An azoospermia factor (AZF) in the distal region of Y chromosome was suggested. The AZF region, was later subdivided into AZFa, AZFb and AZFc. Deletions of the Y chromosome can result in a wide range of anomalies such as Sertoli-Cell-Only-Syndrome (SCOS) or complete absence of germ cells, maturation arrest and hypospermatogenesis. Deletions of Y chromosomes are found mainly in men with azoospermia or oligozoospermia.

CONCLUSION
Cytogenetic studies alone are not enough to find out the exact cause of infertility in men. FISH and PCR also should be done to rule out microdeletions of Y chromosome. The development of assisted reproductive technology (ART), especially ICSI, now offers new hope for the frustrated couple. But the risk of transmitting genetic disorder or infertility to the baby is high in such cases. Appropriate genetic counseling should be offered to the couple before ICSI. Pre-implantation genetic diagnosis (PGD) in combination with ICSI can prevent the transmission of genetic disease to the offspring in these couples

Acknowledgement:
The authors extend their heartfelt gratitude to Dr. K. Jayasree , the Head of the Department of Anatomy, for giving permission to collect data from the Cytogenetic Laboratory. We also thank Dr.Narayanan Govindaraj who gave a lot of contribution in setting up the Laboratory and encouraged us while conducting this study.

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