

KARYOTYPE ANALYSIS IN CASES OF PRIMARY MALE INFERTILITY

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ABSTRACT

Introduction: Infertility is a major health problem in 10-15% Indian couples affecting their psychological and social wellbeing. There is increasing recognition to the contribution of genetic abnormalities to the causation of male infertility. Genetics play a important role in infertility by controlling the physiological processes including hormonal factors, spermatogenesis, and sperm quality. The aim of the study was to find out the Chromosomal abnormalities that play a major role in male infertility cases with non-obstructive azoospermia and oligospermia. The timely investigation to detect genetic abnormality gives better understanding about prognosis to the patients and helps in providing genetic counseling with early intervention, management and also understanding risks involved in transmission of abnormality to future generations.

Materials and methods: In present study total 30 male cases of primary infertility clinically diagnosed and conformed by semen analysis as unobstructed azoospermia and oligospermia were selected. Their karyotypes were prepared and studied for chromosomal abnormalities

Result: The Numerical chromosomal abnormality was found in 2 (6.66%) cases of azoospermic group in the form of Klinefelter syndrome (47,XXY). In cases of oligospermia 1 (3.34%) case had an abnormality in the form of Robertsonian translocation involving 14:15 chromosome. The total 3 cases (10%) were found to have gross chromosomal abnormality by conventional cytogenetic method.

Conclusion: In cases of Klinefelter syndrome (47,XXY) due to altered karyotype or due to meiotic non-disjunction, the residual gametes may be extracted through Testicular / Epididymal Sperm Aspiration (TESA). It is necessary that the diagnosis be made as soon as possible, so as to guarantee the cryopreservation of the semen before complete infertility sets in.

KEY WORDS: Infertility, Karyotypes, Klinefelter syndrome, Azoospermia, Oligospermia.

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INTRODUCTION

Infertility is clinically defined as 'A disease of the reproductive system with the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected intercourse.' So it is an inability to conceive after one year or more of regular unprotected intercourse [1].

A couple that fails to conceive after 12 months of regular intercourse, in the absence of contraceptive use is defined, as infertile couple [2]. Infertility is a major health problem globally affecting approximately 10-15% of the couples [3]. Infertility is grossly subdivided into Primary infertility and Secondary infertility. Primary

infertility is defined as no conception in someone who never had any previous conceptions whereas Secondary Infertility is defined as the inability to conceive after at least one previous pregnancy [4]. The infertility in 50% of these childless couples is attributable to male factors. About 10% genes in Human Genome are related to spermatogenesis [5].

Genetic abnormality accounts for 15-30% cases of male infertility. Genetic factors involved in male infertility may be chromosomal disorders involving Sex chromosomes and Autosomes, Y-Chromosome Micro-deletions, DNA mutations and Endocrine disorders of genetic origin [6]. The most common numerical chromosomal abnormality observed in azoospermic cases of infertility is represented by Klinefelter syndrome (47,XXY). The Micro-deletions of the Y chromosome removing the azoospermia factor (AZF) region are found in men suffering from azoospermia or oligospermia and are the second most frequent genetic cause of spermatogenic failure after Klinefelter syndrome [7].

The Robertsonian translocations and Reciprocal translocations are found more in oligospermic cases (Sperm count less than 15 million/ml) than in azoospermic cases (absence of Sperm) [8]. Understanding of genetic basis of male infertility cases will determine the appropriate treatment plan and prognosis with the use of assisted reproductive technology (ART) like In Vitro fertilization (IVF) and Intra-cytoplasmic sperm injection (ICSI).

MATERIALS AND METHODS

For the present study 30 male cases having primary infertility without any known cause were selected. The cases were undergoing treatment in the Infertility clinic, Department of gynecology, Institute of Kidney diseases, Civil Hospital, Ahmedabad. The approval was obtained from the institute Research council and Ethics committee of Civil hospital, Ahmedabad prior to the commencement of the study. The patients were selected based on their semen analysis. All 30 cases with abnormal semen analysis in the form of Azoospermia and Oligospermia were selected. Karyotype study was done in all 30 male cases having primary infertility. The procedure protocols were followed according to the

guidelines from the book *Rooney and Czepulkowski, Human Cytogenetics: A practical approach* [9]. About 30 metaphase plates were observed in each case and finally a photograph was obtained from a good quality metaphase slide. The chromosomal findings were described according to the international system of Human Cytogenetic Nomenclature using Automatic Karyotyping software. Correlation of chromosomal finding was done with other parameters and with similar studies done in past.

OBSERVATIONS AND RESULTS

Table 1: Semen analysis.

Semen Analysis	Number of cases	Percentage (%)
Oligospermia	16	53.33
Azoospermia	14	46.67
Total	30	100

Table 2: Cytogenetic findings.

Metaphase findings	Number of cases	Percentage (%)
Numerical abnormality	2	6.66
Structural abnormality	1	3.34
Apparent Normal Karyotype	27	90
Total	30	100

Table 3: Interpretation of Karyotype finding.

Karyotype Finding	Interpretation	No of cases	Percentage (%)
Apparently Normal	46,XY	27	90
Abnormal Karyotype	47,XXY (Klinefelter syndrome)	2	6.66
	45, XY t (14:15) (Robertsonian translocation)	1	3.34

Table 4: Karyotype finding according to sperm concentration.

Karyotypes	Oligospermic (n=16)	Percentage (%)	Azoospermic (n=14)	Percentage (n=14)
47,XXY	0	0	2	14.28
45,XY, t(14:15)	1	6.25	0	0
46,XY	15	93.75	12	85.72
Total	16	100	14	100

The male cases of primary infertility with non-obstructive Oligospermia and Azoospermia who came for the infertility treatment were selected. Following observations were noted after relevant history, laboratory investigations and cytogenetic study in 30 such cases. According to their semen analysis 16 (53.33%) cases were classified in to Oligospermic group (Sperm

count <15 million/ml) and 14 (46.67%) cases were classified into Azoospermic group. (TABLE-1) Out of the 30 cases studied total 3(10%) cases showed constitutional chromosomal abnormality. The Numerical abnormality was found in 2 (6.66%) cases and the structural abnormality was found in 1 (3.34%) case. (TABLE-2) Table shows the interpretation of the karyotype study. The Numerical abnormality was found in 2(6.66%) cases in the form of 47, XXY KS genotype and structural abnormality in the form of Robertsonian translocation with genotype 45,XY, t (14:15) was found in 1 (3.33%) case. In rest of the 27(90%) cases the karyotype was apparently normal in conventional cytogenetic method. (TABLE-3) Out of total 16(100%) Oligospermic cases, 1(6.25%) case had a karyotype abnormality in the form of Robertsonian translocation 45, XY, t(14:15) and 15 (93.75%) of the Oligospermia cases the karyotype was found to be normal. Out of total 14 (100%) cases of Azoospermia, the numerical abnormality in the form of 47,XXY (KS) was found in 2 (14.28%) cases whereas in 12 (85.72%) cases of Azoospermia the karyotype study was found to be normal. (TABLE-4)

DISCUSSION

An attempt was made to find out chromosomal abnormalities in cases of male infertility and to determine the types of chromosomal abnormalities that play a major role in the causation of infertility in oligospermia and azoospermia. In the study of semen analysis it was found that out of 30 cases 16 (53%) cases belong to Oligospermia and 14 (47%) cases belong to non-obstructive azoospermia. According to the semen analysis of 88 primary infertile men in the study of Nagvenker P, Desai K, 46(52%) cases belong to oligospermia and 42(48%) of the cases were of azoospermia who had undergone cytogenetic analysis prior to ICSI treatment [10]. Prior to the cytogenetic analysis conducted by MT Akbari et al. in 222 male cases of primary infertility. The semen analysis had azoospermic group included 132 (59.44%) which was higher than 90(40.54%) cases oligospermic group [11]. Whereas study conducted by Ceylan GG et al. out of 90 infertile men with primary infertility found equal number of cases that is 30 (33.3%)

cases belong to non-obstructive azoospermia, 30 (33.3%) men with oligospermia 30 (33.3%) [8].

In the present study the highest incidence (6.66%) of Numerical chromosomal abnormality was found in azoospermic group in the form of Klinefelter syndrome (47,XXY) and no structural abnormality was found in sex chromosomes. In cases of oligospermia, 3.34% cases had an abnormality in the form of Robertsonian translocation involving 14:15 chromosome. The total 3 cases (10%) were found to have gross chromosomal abnormality by conventional cytogenetic method. The present study showing the 10% prevalence as a genetic cause (structural and numerical chromosomal abnormality) for "non-obstructive azoospermic and oligospermic cases of male infertility is supporting the literature showing overall prevalence of chromosomal anomalies in infertile men is approximately 10%.¹² It is also supporting the evidence that chromosomal abnormality is inversely related to sperm concentration. The prevalence found in the present study in Azoospermia cases is 14.28% and 6.25% in oligospermic men. Cytogenetic analysis carried out by Gupta Archana observed that 10.9% subjects had chromosomal abnormality. In azoospermia group, seven subjects (15.7%) had chromosomal abnormalities. All these subjects had numerical chromosomal abnormalities. 4 cases showing 47, XXY karyotype while 3 had 46, XY/47, XXY mosaic karyotype. In Oligospermia group 3 subjects (6.6%) were found to have chromosomal abnormalities in the form of Robertsonian translocation anomaly 45, XY, t (14:15) [13]. In the study conducted by Akgul M et al.¹⁴ among 86 azoospermic cases, they found that 15(17.44%) had abnormal karyotype in the form of 47,XXY and mosaic Klinefelter form and among 68 oligospermic cases 5 (6.85%) had abnormal karyotype in the form of autosomal translocations [14]. In 222 infertile male study, M T Akbari et al. observed that the overall chromosome abnormality rate was 13.96%. The numerical chromosomal alterations were found in 27 (20.4%) azoospermic males in the form consisting of 25 (18.9%) having numerical abnormality in the form of 47(XXY) and 2 (1.5%) cases showed structural abnormality.

Among the 90 oligospermic males, both kinds of chromosome abnormalities were seen in equal number of 2 patients (2.2%) [11].

In cases of Klinefelter syndrome (47,XXY) due to altered karyotype or due to meiotic non-disjunction with residual spermatogenesis, there are some techniques of assisted fertilization, which may provide these patients the possibility of having children of their own. If some immature gametes are present in the testes, more specifically in the seminiferous tubules, these may be extracted through multiple testicular biopsies by means of testicular sperm extraction (TESA). The probability of finding spermatozoa with this method is between 25 to 40% and it is fundamental that the diagnosis of KS be made as soon as possible, so as to guarantee the cryopreservation of the semen before complete infertility sets in [15].

The similar cases of non-obstructive azoospermia and oligospermia other than Klinefelter syndrome can be possibly treated with advance assisted reproductive techniques like IVF and ICSI conditionally they are all treated as cases of Y chromosome Micro-deletion and should be screened using more genetic techniques like Florescence in-situ hybridization (FISH) and or Comparative genomic hybridization (CGH). If half of all above mentioned cases undergo ICSI, the incidence of male infertility will double in seven generations [16]. Therefore, it has been recommended that all males having AZF deletions should undergo andrological examination at puberty and if sperms are present; these should be cryopreserved in early adulthood before their possible decline with age. To prevent the transmission of cytogenetic abnormalities into the subsequent generations it is now a days recommended that all the cases of IVF or ICSI should undergo Embryo biopsy and Pre-implantation genetic diagnosis (PGD) using fluorescence in situ hybridization (FISH) or other sophisticated techniques to evaluate chromosomal composition which can determine the normal embryos suitable for transfer [17].

CONCLUSION

The evidence is suggestive that semen analysis determines the case selection to find out the exact cause of male infertility in majority of the

cases. The prevalence of apparent genetic abnormality with conventional karyotyping is 10% in the present study and is supporting strongly the overall prevalence of male infertility due to genetic cause. The Numerical chromosomal abnormality found in present study is 6.66% and structural chromosomal abnormality found is 3.34% are also supporting strongly with the average percentage found as a genetic factor in similar studies. The Klinefelter syndrome (47,XXY) found as a part of numerical chromosomal abnormality is in 6.66% cases, which is found to be a cause of male infertility in the range of 5-10% as observed in similar studies. The cases of Klinefelter syndrome were recommended to go for TESA to find any residual spermatozoa, and if found they should be cryopreserved for further advanced treatment options like ICSI. The cases with structural chromosomal abnormality and apparently normal karyotypes by conventional cytogenetic methods were counseled for genetic screening by sophisticated genetic investigation like FISH or CGH to find out micro-deletion in Y chromosome. All the cases of study group should be counseled for possible pre-implantation genetic diagnosis (PGD), which are undergoing treatment with IVF or ICSI

ABBREVIATIONS

KS - Klinefelter syndrome

TESA - Testicular Epididymal Sperm Aspiration

AZF - Azoospermia factor

ART - Assisted reproductive technology

IVF - In Vitro fertilization and

ICSI - Intra-cytoplasmic sperm injection

FISH - Florescence in-situ hybridization

CGH - Comparative genomic hybridization

PGD - Pre-implantation genetic diagnosis

Conflicts of Interests: None

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