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Chromosomal Abnormalities of Infertile Men in a Tertiary Care Teaching Hospital

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Abstract: Infertility affects 1 in 6 couples worldwide and among them males contributes 50% of cases. Male infertility due to genetic factors accounts upto 15%–30% cases. The aim of this study is to determine the frequency and type of chromosomal abnormalities in infertile men. It is a cross sectional academic research environment study. 37 Infertile men with azoospermic (24), oligoazoospermic (6), oligoasthenoteratospermic (7) but otherwise apparently healthy were selected for the study. Peripheral blood lymphocytes were obtained for karyotyping and metaphases were studied by standard GTG banding procedure. The common Chromosomal abnormality of Klinefelter syndrome - 47,XXY was observed in 4 (10.8%) patients and balanced carrier translocation of 46,XY,t(13;19)(q12;q13.4) was observed in 1 patient and 46,XY,t(7;9)(q11.2;p13) in another phenotypically normal patients (5.4%). A normal chromosomal polymorphic variant of 15ps+ was observed in 2 cases and 15pstk+ps+ in one case. This study concluded that the karyotyping is mandatory for the infertile men as diagnostic establishment and suitable genetic counseling, for those seeking assisted reproductive technologies (ART).

Keywords: Male infertility, chromosomal abnormalities, GTG banding, Azoospermia, Oligospermia

INTRODUCTION

Infertility is a communal health problem in India, which affects approximately 15% of married couples in their reproductive age [1]. Since male factor contribute about 50% of the cases [2], should be evaluated concurrently with women. Although it's not a life threatening condition but it has the social stigma in certain societies. Genetic factors are recognized as an important detrimental factor in the etiology of male infertility, which affect 30% of individuals taking treatment at infertility clinics [1].

Chromosomal abnormalities were diagnosed in 3–19% of infertile men [3] with both sex chromosomal and autosomal alterations. Previous studies shown that genetic alteration including aneuploidy, translocations, inversions, deletions of the Y chromosome and DNA damage may be an effective cause in infertility [4]. The main intention of this study was to find out the frequency and types of chromosomal abnormalities in male infertility patients.

MATERIALS AND METHODS

Patients

This investigation was conducted with the approval of our Institutional ethics committee of Nitte University in accordance with the revised declaration of Helsinki [5]. The prior informed written consent form was collected from all the participants. A detailed clinical history, family history, reproductive problems and occupation was recorded for each patient. Physical examination was conducted in order to categorize anatomical problems. Semen analysis also was performed according to the World Health Organization (WHO) Guidelines. A total of 37 infertile men with azoospermia (n=24), Oligospermia (n=6) and Oligoasthenoteratospermia (n=7) were included in the study.

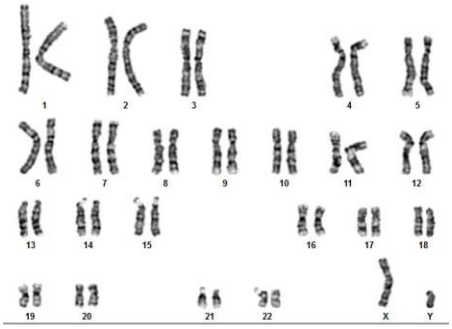
Methods

Karyotype analysis was performed using the standard GTG banding procedure with slight modifications [6]. 2ml of Peripheral blood from each patient was collected in Sodium heparinized vacutainer for chromosomal analysis. Cytogenetic analysis was performed in KSHEMA Centre for Genetic Service, Central Research Laboratory, K. S. Hegde Medical Academy, Deralakatte, and Mangalore. A 72 hours lymphocytes culture was setup with 8ml of PB Max medium (Gibco by *life* technologies™) and 200µl Phytohemagglutinin (PHA) (M form) (Gibco by *life* technologies™). Culture flask was incubated horizontally in CO2 incubator at 37˚C for 72 hours. 45µl of KaryoMAX COLCEMID (10µg/ml) (Gibco by

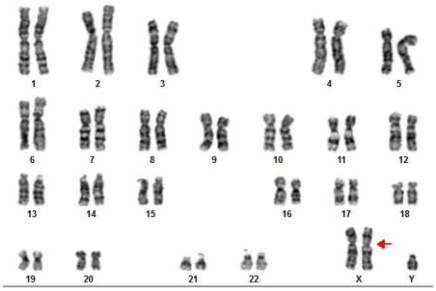
life technologies™) was added at 68th hour to arrest the cells at the metaphase stage and mixed. After 45 minutes of incubation the culture was transferred into the sterile 15ml centrifuge tube and spun at 2000rpm for 10 minutes. Then the cells were treated with the hypotonic solution (0.075M KCl) for 13 minutes at 37˚C and fixed with Carnoy's fixative (3:1 ratio of Methanol and Glacial Acetic Acid). The cell pellet suspension was dropped on prechilled slides and dried at 45˚C. Then the slides were aged at 60˚C in a dry oven for overnight. Next day slides were treated with standard Trypsin (1:250) (Gibco by *life* technologies™) solution and stained in 1% Giemsa (Merk) solution. A minimum of 20 well spread metaphases were analysed using Olympus BX53 microscope. 5 good quality metaphase spreads with 350-550 band resolution were captured using the CCD camera attached with microscope. Analysis and karyogram of the each metaphase was performed using the GEN*ASI*S software. Karyotypes were interpreted according to the ISCN (2013) [7].

RESULTS

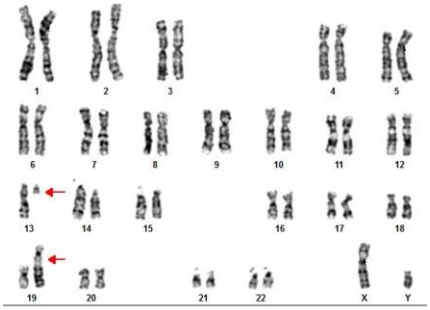
Normal chromosome complement (Figure 1) was detected in 31 (83.8%) of the 37 patients analyzed. Structural and numerical chromosomal abnormalities were observed in 6 patients (16.2%). Among them the most prevalent chromosomal abnormality of Klinefelter syndrome (47,XXY) (Figure 2) was detected in 4 (10.8%) patients and balanced carrier translocation was observed in 2 (5.4%) patients. Among the balanced translocation, one azoospermic patient showed the carrier balanced translocation between chromosome 13 and 19 – 46,XY,t(13;19)(q12;q13.4) (Figure 3) and one oligospermic patient with the carrier balanced translocation between chromosome 7 and 9 – 46,XY,t(7;9)(q11.2;p13) (Figure 4) (Table 1). A normal chromosomal variant of 15ps+ was observed in 2 cases and 15pstk+ps+ (Figure 5) in one case (Table 2).



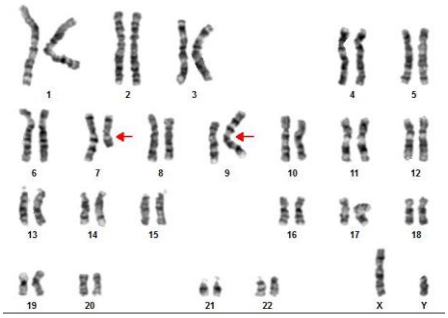
Fig– 1: Normal chromosome complement – 46,XY



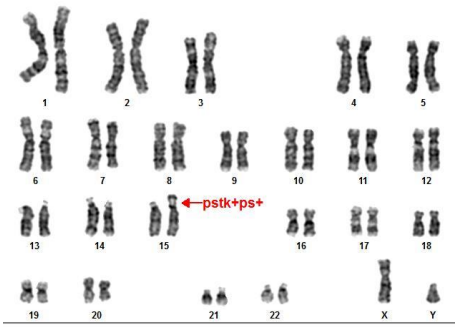
Fig– 2: Male with Klinefelter syndrome – 47,XXY



Fig– 3: Male karyotype with balanced carrier translocation between chromosome 13 and chromosome 19 - 46,XY,t(13;19)(q12;q13.4)



Fig– 4: Male karyotype with balanced carrier translocation between chromosome 7 and chromosome 9 - 46,XY,t(7;9)(q11.2;p13)



Fig– 5: Male karyotype with normal polymorphic variant of 15pstk+ps+ - 46,XY,15pstk+ps+

Table–1: The Observed chromosomal abnormalities:

	Karyotype	**No of Case**
No chromosomal abnormalities	46,XY	31
Numerical Abnormalities	47,XXY	4
Structural abnormalities - Translocation	46,XY,t(13;19)(q12;q13.4) 46,XY,t(7;9)(q11.2;p13)	1 1

Table–2: Chromosomal Polymorphic Variants

Polymorphic Variants	No of Patients	Karyotype
15ps+	1	46,XY,15ps+
15pstk+ps+	1	46,XY,15pstk+ps+

DISCUSSION

The last 20 years of research has indicated that various chromosomal abnormalities can cause spermatogenic breakdown at various points, consequently resulting in chromosomally derived infertility. Several studies have shown a high incidence of chromosomal abnormalities in infertile men, ranging from 2.2% to 14.3% with an overall incidence of 7.1% and also reported that its occurrence is higher in azoospermic men with sex chromosome abnormalities than oligozoospermic patients with frequent autosomal abnormalities [8].

Chromosomal analysis plays an important role in determining the cause of Klinefelter's syndrome characterized by tall stature, gynecomastia, testicular atrophy, azoospermia or oligospermia and sterility with the prevalence of 0.1% in general population [9]. It is the most common karyotypic abnormality in severe male factor infertility, affecting 7%–13% of azoospermic men [1]. In the present study, we have observed 4 azoospermic men with Klinefelter syndrome – 47,XXY. The extra X chromosome of Klinefelter syndrome influences the mechanism of spermatogenesis, which affects testicular development, Leydig cell insufficiency, Sertoli and Leydig cells apoptosis regulation [10].

Okada H *et al.* used the zona-free hamster oocytes penetration technique for meiotic study and reported that a significantly increased rate of sex-chromosome hyperploidy such as 24,XX or 24,XY than a 23,X or 23,Y karyotype in 92% of sperm nuclei from a patient with 47,XXY karyotype [11]. Therefore, we can speculate that germ cells in patients with

Klinefelter's syndrome have the potential to increase the incidence of sex chromosome hyperploid spermatozoa. Klinefelter syndrome has been recently reported to be associated with osteoporosis and increased mediastinal cancer risk among the infertile men [12].

Pericentric inversion of chromosome 9, inv (9) (p11q12)/inv(9)(p11q13) is a common chromosomal rearrangement in infertile men without any phenotypic effect and some cytogeneticists consider it as a normal variant [13]. Heterochromatic variations like qh+ and inversions of chromosomes 1 and 9, and short arm of acrocentric chromosomes especially 15ps+ and 22ps+ observed significantly higher in couples with bad obstetric history [14]. When compared with normal karyotype, polymorphic variants revealed decreased ability of the spermatozoa to penetrate hamster oocytes [15]. In the present study, we have identified polymorphic variant of pseudo satellites on chromosome – 15 (15ps+) in two infertile men and 15pstk+,15ps+ in one male with azoospermia.

The occurrence of translocation is more familiar in infertile males than normal males [3]. Carriers of these translocations usually have normal phenotype but may leads to recurrent pregnancy loss, chromosomally abnormal offspring with mental retardation, congenital malformation and development delay. Chromosome pairing of infertile men with chromosome translocations during meiosis could be very sensitive and consequently making match between homologous chromosomes is difficult. Eventually, turbulence in chromosome segregation during spermatogenesis might leads to spermatogenic arrest [16].

Autosomal balanced translocations in males have an increased risk of oligospermia and the chromosomal studies on their spermatozoa showed an unbalanced karyotype in variable proportions [17]. In the present study, balanced carrier translocation between chromosome 13 and 19 – 46,XY,t(13;19)(q12;q13.4) in one azoospermic patient and one oligospermic patient with the carrier balanced translocation between chromosome 7 and 9 – 46,XY,t(7;9)(q11.2;p13) was observed. Cases of infertility with different translocations of chromosome 9 were also reported; t(9;11), t(9;13), t(9;3), t(7;9), t(2;9), t(4;9) [4].

Ravel C *et al***.** studied 10202 sperm donors with known karyotypes shows that the frequency of chromosomal aberrations is not influenced by a previous normal fertility or by an uneventful familial history when compared to that found at birth [18]. Infertility cases with balanced translocations of different chromosomes were reported; t(1;19), t(3;13), t(1;9), t(9;10), t(9;3), t(1;4), t(7;8), t(3;6), t(1;11), t(1;10), t(3;18), (7;8); t(7;14), t(7;17), (13;19), t(6;17) [13,18, 19,20]. Incorrect chromosome coupling and crossing over in meiosis is the common results of these chromosomal abnormalities findings. Eventually, the other possibility in chromosomal break points is the exclusion of the genes, related to testicular development and function.

However, this data emphasizes that the authentic frequency of chromosome abnormalities needs to be investigated with a larger size of sample.

CONCLUSION

The common Chromosomal abnormality of Klinefelter syndrome – 47,XXY, balanced carrier translocation of 46,XY,t(13;19)(q12;q13.4), 46,XY,t(7;9)(q11.2;p13) and normal chromosomal polymorphic variant of 15ps+, 15pstk+ps+ was observed in this study. Chromosomal analysis is not cost effective in this group of subjects in view of low prevalence of aberrations. Genetic testing can make an important contribution in the treatment of patients planning to Intracytoplasmic sperm injection (ICSI) or testicular sperm extraction (TESE)/ICSI treatment. This data demonstrates that karyotyping is mandatory for the infertile men as diagnostic test and for the suitable genetic counseling. It is important to know whether there is a genetic cause of male infertility with the above criteria for those seeking assisted reproductive technologies (ART).

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