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Research Article

Klinefelter's syndrome in azoospermic infertile males of Vidarbha region, Central India

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ABSTRACT

Background: Infertility was defined as involuntary failure of conception in a married couple after 1 year of regular unprotected intercourse. Male factor infertility is a distressing condition that adds to the psychological trauma to majority of couples. The widely accepted methods of screening for infertility in males are semen analysis and cytogenetic studies. Klinefelter syndrome, with an incidence of 1:600 male newborns, is the most frequent form of male hypogonadism.
Methods: 30 non-obstructive azoospermic infertile males were selected for present study. For each subject, chromosomal analysis was carried out by conventional as well as giemsa trypsin giemsa (GTG) technique in cytogenetic laboratory. Total 25 metaphases i. e. 15 conventional and 10 G-banded metaphases were analyzed, in each case. In cases with chromosomal abnormalities, total 45 metaphases i. e. 25 conventional and 20 G-banded metaphases were studied. Selected metaphases were photographed using CCD camera.
Results: Three subjects had a chromosomal count of 47 in all the metaphase studied. The additional chromosome was closely matching with the X chromosome. Hence the karyotype showed numerical aberration with an extra 'X' chromosome i. e. 47, XXY suggestive of Klinefelter's syndrome. This was confirmed by G-banding. All of the three subjects had bilateral testicular atrophy and one had typical features of Klinefelter's syndrome except gynecomastia.
Conclusions: On cytogenetic analysis of 30 azoospermic infertile subjects, chromosomal abnormality of 47, XXY (Klinefelter's syndrome) was found in 3 subjects. The total percentage of Klinefelter's syndrome in present study comes to 10%.

Keywords: Azoospermia, Metaphase, Chromosome, Klinefelter syndrome

INTRODUCTION

Infertility was defined as involuntary failure of conception in a married couple after 1 year of regular unprotected intercourse. A couple was called primary infertile when wife never conceived and secondary infertile or subfertile when wife had conceived at least once irrespective of outcome of pregnancy, but failed to have successive pregnancies (WHO, 2000).[1]

Male factor infertility is a distressing condition that adds to the psychological trauma to majority of couples. Infertility affects about 15% of all couples attempting pregnancy, with male factor identified in approximately half the cases by Ken McElveavey et al in 1999.[2]

According to De Krester one of the major contributing factors of failure of sperm production in testis is genetic disturbance.[3] This can be seen either at chromosomal level or at gene level. Chromosomal abnormalities can occur in somatic cells (mitotic), testicular germ cells (meiotic) or spermatozoa (gametic). In either of these it could be numerical or structural, involving either sex chromosomes or autosomes.

From the time of earliest records in every culture there has been reference to infertility and in every culture there are prayers or ceremonies to try to ensure fertility (TB Hargreave).[4] Infertility is globally accepted as one of the major problem in family welfare programmes. Evaluation of infertile couple necessitates simultaneous assessment of both the partners. The widely accepted methods of screening for infertility in males are semen analysis and Cytogenetic studies. Klinefelter HF in 1942 describe 9 infertile males who had breast development, small testicles with no sperm in their semen.[5] Barr M et al, demonstrated Barr body in the buccal smears of 10 cases of infertile males and labelled them as Klinefelter's syndrome.[6]

Klinefelter syndrome, with an incidence of 1:600 male newborns, is the most frequent form of male hypogonadism. However, despite its relatively high frequency, the syndrome is often overlooked by Kamischke A et al.[7] Clinically, the syndrome is characterized in adolescents and adults by the constellation of small, firm testes and symptoms of androgen deficiency. Other often-associated clinical features are azoospermia, tall stature, and bilateral painless gynecomastia. Diagnosis is confirmed by chromosome analysis performed in lymphocytes by Jacobs and Strong.[8]

METHODS

30 non obstruction azoospermic infertile males from AVBR Hospital, Sawangi (Meghe), Maharashtra, India were selected for present study. These subjects were referred for chromosomal analysis after medical checkup to rule out varicocele, hernia, genital tuberculosis, pulmonary and extra pulmonary tuberculosis, venereal diseases, endocrinal disorders and abnormal testicular biopsy.

Written consent was obtained from each subject and 3 ml venous blood was collected in a sterile bulb with the help of preheparizined syringes. Chromosomal analysis was carried out by conventional as well as giemsa trypsin giemsa (GTG) technique in cytogenetic laboratory, department of Anatomy, Jawaharlal Nehru Medical College, Sawangi (Meghe), Wardha, Maharashtra, India.

All slides were screened first under low power objective of microscope and then under oil-immersion objective. For each subject, total 25 metaphases i. e. 15 conventional and 10 G-banded metaphases were analyzed. In cases with chromosomal abnormalities, total 45 metaphases i. e. 25 conventional and 20 G-banded metaphases were studied. Selected metaphases were photographed using CCD camera.

RESULTS

On cytogenetic analysis of 30 azoospermic infertile subjects chromosomal abnormalities in the form of 47, XXY (Klinefelter's syndrome) were found in 3 subjects. Hence the total percentage of Klinefelter's syndrome (47, XXY) in present study comes to 10% (Table 1).

Table 1: Chromosomal abnormalities in present study.

Seminal feature	No. of subjects	Subjects with normal karyotype (46, XY)	Subjects with Klinefelter's syndrome (47, XXY)	Abnorm-ality (%)
Azoospermia	30	27	3	10

Conventional method

Three subjects had a chromosomal count of 47 in all the metaphase studied (Table 2). The additional chromosome was medium sized submetacentric chromosome belonging to group C, which was closely matching with the X chromosome. Hence the karyotype showed numerical aberration with an extra 'X' chromosome i. e. 47, XXY suggestive of Klinefelter's syndrome. This was confirmed by G-banding (Table 3). Remaining 27 (90 %) subjects had normal chromosomal count of 46, XY in all the metaphases.

Table 2: Observations of conventional method.

No. of cases	Numerical abnormalities	%
03	Extra chromosome belonging to 'C' group, resembling 'X' chromosome	10

Table 3: Observations on G-banding.

No. of cases	Numerical abnormalities	Karyotype
03	Two 'X' chromosomes belonging to group 'C' confirmed.	47, XXY

GTG method

The findings of conventional method were confirmed by G-banding (Table 3).

03 subjects had an additional chromosome in group 'C' resembling 'X' chromosome (conventional method, Table 2). This additional chromosome was confirmed by G-banding as X chromosome (Figure 1 and 2). Hence numerical chromosomal anomaly of 47, XXY, which is diagnostic of Klinefelter's syndrome, was confirmed in 03 subjects (Table 3).

The clinical features and physical findings in these subjects are shown in Table 4.

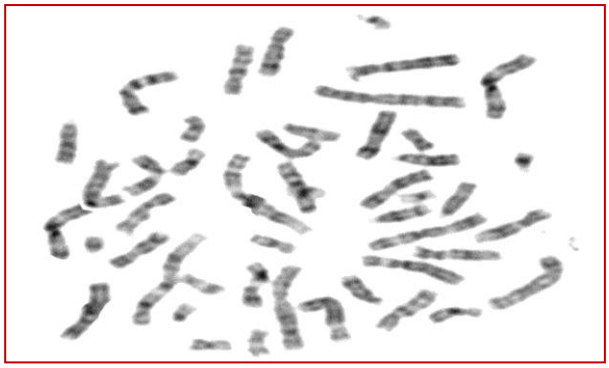

Figure 1: Metaphase spread of 47, XXY showing two 'X' chromosomes and single 'Y' chromosome.

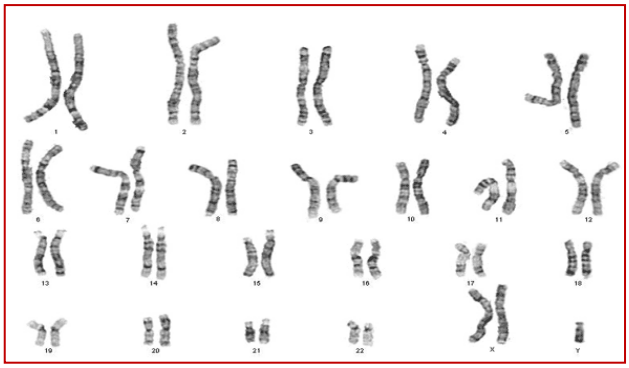

Figure 2: Karyotype of 47, XXY (Klinefelter's syndrome).

Table 4: Clinical features and physical findings in subjects with Klinefelter's syndrome (47, XXY).

Age in (years)	Occupation	Habits	External genitelia	Gonads	Semen status	Karyo	Other relevent findings
27	Labourer	Nil	Small size penis, scanty pubic hairs	Testes in scrotum, shows B/L testicular atrophy	Azoo.	47,XXY	Ht.-162cm, secondary sex charecters partially developed
30	Driver	Alc & Tob	Micropenisvery scanty pubic hairs	Testes in scrotum, shows B/L testicular atrophy	Azoo.	47,XXY	Ht.-170cm, Beard and mustach, chest hairs and axillary hairs absent.
35	contractor	Alc & Smo	Normal	Testes in scrotum, shows B/L testicular atrophy	Azoo.	47,XXY	Ht.-160cm, secondary sex charecters partially developed

Azoo- Azoospermic; Alc- Alcohol, B/L- Bilateral; Tob- Tobaco; Smo- Smoking; Karyo- Karyotype

DISCUSSION

In the present study an attempt has been made at rural hospital level to find out incidence of Klinefelter's syndrome in azoospermic infertile males. They were referred from AVBRH Sawangi (Meghe), Wardha, India after semen analysis and medical and surgical check-up to rule out any obstructive lesions in the reproductive tract.

In present study semen analysis of the subjects were done before they were referred for chromosomal analysis. The criteria to call a subject as azoospermic was according to guidelines recommended by WHO (WHO Manual, 2000).[1]

In present study, for each subject, total 25 metaphases i. e. 15 conventional and 10 G-banded metaphases were analyzed. In cases with chromosomal abnormalities, total 45 metaphases i. e. 25 conventional and 20 G-banded metaphases were studied. This screening protocol was similar to that used by most of the workers. Peter JJ et al studied total 30 metaphases with addition of some QFQ-banded metaphases when they got Y chromosomal abnormalities.[9] Chandley AC analyzed up to 30 GTG, CBG and QFQ banded metaphases in each subject and R Ghosh R et al analyzed 10 GTG and 10 QFQ banded metaphases in each case.[10,11] Tarnekar et al studied 15 conventional metaphases and 10 G-banded metaphases in each subject and in cases with 'Y' chromosomal abnormalities 15 additional 'C' banded metaphases (total 40).[12] In the study of Mohan Rao M et al 30 metaphases were counted in each case without any banding

technique.[13] In cases of chromosomal abnormalities they increased the study of metaphases up to 100.

On cytogenetic analysis of 30 azoospermic infertile subjects chromosomal abnormality of 47, XXY (Klinefelter's syndrome) was found in 3 subjects. The total percentage of Klinefelter's syndrome in present study comes to 10%.

The proportion of chromosomal abnormalities in azoospermic subjects in various studies were as follows, Rao MM et al 14.28%; Ghosh R et al 13%, Chandley AC et al 15.32% and Tarnekar et al 14.81%, Rao L et al 14.42%, Nagvekar et al 14.29% findings of these studies were matches with present study (10%).[10-15] But the findings of Peter JJ et al of 20.8% and Gunduz G et al of 34.1% are much higher than our report.[9,16] Probably they might have selected highly suspected cases of chromosomal abnormalities on the basis of clinical features.

The numerical chromosomal abnormality observed in present study was Klinefelter's syndrome (47, XXY) in 3 of 30 azoospermic subjects (10%). This observation correlates with findings of percentage of Klinefelter's syndrome cases in azoospermic subjects of various other workers (Table 5); Chandley AC et al, 12.9%, Mohan Rao M et al, 14.28%; Peter JJ et al, 16.6%; Ghosh R et al, 13%; Gunduz G et al, 19.5% and Tarnekar et al of 7.4%.[9-13,16]

Oligozoospermic subjects are seldom found with Klinefelter's syndrome. However studies carried out on larger number of oligozoospermic subjects as stated below, could detect cases of 47, XXY or mosaic karyotypes. Taking only those oligozoospermic subjects who had sperm count below $10x10^6$/ml, Retief AE et al and Bourrouillou G et al found 7 out of 390 (1.79%) and 4 out of 569 (0.70%) cases (inclusive of mosaics) of Klinefelter's syndrome respectively (Table 5).[17,18]

Table 5: Percentage of Klinefelter's syndrome cases in various studies.

Reported by	In Azoo		In Oligo		In Asp		Overall %
	No.	%	No.	%	No.	%	
Chandley AC et al[10] (n=416)	16/124	12.9	0/292	0	--	--	3.84
M Rao et al[13] (n=117)	9/63	14.28	0/54	0	--	--	7.69
Peter JJ et al[9] (n=96)	4/24	16.6	0/53	0	3/19	15.7	7.29
Retief AE et al[17] (n=496)	11/106	10.37	7/390	0.7	--	--	3.62
Bourrouillou G et al[18] (n=952)	49/383	12.79	4/569		--	--	5.56
Ghosh R et al[11] (n=102)	3/23	13.04	0/65	0	1/14	7.14	3.92
Gunduz G et al[16] (n=102)	8/41	19.5	0/61	01.79	--	--	7.84
Tarnekar et al[12] (n=60)	2/27	7.4	0/30	0	0/3	0	3.33
Present study	3/30	10	-	-	-	-	10

Azoo : Azoospermic; Oligo :Oligozoospermic; Asp :Aspermic.

Few available literatures show aspermic subjects with Klinefelter's syndrome. Peter JJ et al and Ghosh R et al have reported 15.7% and 7.14% cases of Klinefelter's syndrome in aspermic (Table 5).[9,11]

Retief AE et al in South Africa and Bourrouillou G et al in France worked with large number of azoospermic and oligozoospermic cases (496 and 952 respectively) and found overall 3.62% and 5.56% cases of Klinefelter's syndrome respectively.[17,18] The total percentage of Klienfelter's syndrome cases (10%) in present study was much higher probably because we had taken only azoospermic cases. Faed et al inferred that there is either a real difference in prevalence of Klinefelter's syndrome cases in different geographical areas or there is a difference in the number of such cases coming for their evaluation.[19]

Though gynaecomastia has been mentioned as a constant feature in Klinefelter's syndrome.[23] In a study of 148 Klinefelter's syndrome cases Okada H et al found only 12.4% subjects had gynaecomastia.[20] Tarnekar et al not reported gynaecomastia in cases of Klinefelter's syndrome.[12] This finding is similar to that of present study as gynaecomastia was absent in all the 3 cases of Klinefelter's syndrome.

The abnormal karyotypes of 47, XXY that we found in present study was amongst the most commonly reported numerical abnormality of sex chromosomes (Koulischer, Schoysman and Micic M et al).[21,22] However different types of autosomal abnormalities were also reported in literatures of male infertile subjects. Amongst the structural rearrangements of autosomes frequently reported were Robertsonian translocations involving chromosomes of group D and G and reciprocal translocations (Chandley AC et al), pericentric inversions (Micic M et al) and deletions (Gunduz G et al).[10,16,22]

Cytogenetic studies of infertile males suggest that the chromosomal abnormalities do formulate a basis of disturbed spermatogenesis. At times the biochemical

process of spermatogenesis is faulty because of an abnormal gene product. Such cases are being increasingly detected with molecular genetic methods such as polymerase chain reaction (PCR) and 'fluorescent in situ hybridization- FISH' etc. Genes controlling spermatogenesis have been located on 'q' arm of Y chromosome (Elreavey et al, Foresta C et al).[23,24]

Several non genetic factors play a role in disturbing the process of spermatogenesis in testis. Hormonal imbalances, exposure to toxic chemicals, ionic radiations, heat exposure, cytotoxic medications and sometimes psycho-social factors have also been identified amongst such factors (Sharpe; Belsey and Helenware).[25]

27 of 30 subjects did not show any chromosomal abnormalities. However prolong exposure to heat has been inculcated as a cause of infertility in people working in metal, glass and ceramic industries and those in sedentary jobs requiring prolonged sitting (drivers, computer operators etc.).

CONCLUSION

3 subjects (10%) were detected with Klinefelter's syndrome which compares favorably with literatures on the same subject.

Assisted method of reproduction was an option of treatment for infertile males. But because of possibilities of chromosomal abnormalities in such cases the specialist undertaking procedures like ICSI, should investigate such subjects properly, including cytogenetic studies. The same study could be combined with molecular genetic studies to ascertain the chromosomal anomalies at molecular level and thereby proper counseling could be given to infertile couples. This can be of vital role in planning of parenthood.

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