Original Article

An Analysis of Cytogenetic and Clinical Phenotype of Klinefelter Syndrome Over 17 Years

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Abstract

Background: *Clinical phenotype in Klinefelter syndrome (KS) shows utmost contrariety according to the genetic presentation. The karyotype 47, XXY is one of the commonest types of sex chromosomal abnormality in males presenting with infertility, hypogonadism, small penis, gynaecomastia and tall stature. Cytogenetic study is the only way to differentiate between chromosomal abnormality and other androgen deficiency disorders. The aim of this study was to investigate cytogenetic and phenotypic profile of Klinefelter syndrome in a group of referred patients with suspected genetic disorders.*

Methods: *This observational study was carried out at the Cytogenetic Laboratory of the Department of Immunology BIRDEM General Hospital for a period of seventeen years from 2000 to 2016. A total of 9,216 patients suspected for different chromosomal abnormalities (e.g. numerical chromosomal disorders, primary amenorrhoea, ambiguous genitalia etc.) were included in this study referred by physicians of various discipline from different areas of Bangladesh. From the patients referred for cytogenetic study, detailed family history and physical findings were noted. Complete genetic examination and pedigree construction was done to exclude non-chromosomal causes of anomalies. For cytogenetic analysis, peripheral lymphocyte culture by the standard method using the G-banding technique was employed.*

Results: *In this study 1.67% (154) of referred patients were diagnosed as Klinefelter syndrome in cytogenetic study and most of them were diagnosed in their adulthood between 20-29 years of age. Classical cytogenetic form of KS-47, XXY (87%) were most common followed by other mosaic and supernumerary X chromosome aneuploidy. Most of the patients presented with tall stature (61.7%) followed by other features such as gynaecomastia (45.5%), eunuchoid skeleton (29.8%), sexual dysfunction (34.41%), small penis (22.7%), and delayed development of secondary sex characteristics (22.7%).*

Conclusion: *Diagnosis of Klinefelter syndrome in early age before puberty is needed to be differentiated from other related disorders and thereby improving the quality of life by providing appropriate and timely treatment. Therefore, we have to focus to improve our overall capacity to diagnose genetic disorders for proper intervention.*

Key words: *Klinefelter Syndrome.*

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Introduction

Klinefelter syndrome (KS) 47, XXY was first described 70 years ago in 1942 by Dr. Henry Klinefelter and he published a report on nine men who had enlarged breasts, sparse facial and body hair, small testes, and an inability to produce sperm.[1] It is one of the commonest congenital chromosome disorders resulting in genetically-determined infertility and hypogonadism with an incidence of 0.1% to 0.2% of male neonates.[2]

KS usually occurs randomly which is not inherited from one's parents. An older mother may have a slightly increased risk of a child with KS.[3] Main mechanisms to

be Klinefelter syndrome is at least one extra X chromosome in addition to a Y chromosome such that there is a total of 47 or more chromosomes rather than the usual 46.[4] The extra chromosome is maintained because of a nondisjunction event during paternal or maternal meiosis I (gametogenesis). Nondisjunction occurs when homologous chromosomes, either X and Y or two X sex chromosomes, fail to separate, producing a sperm with an X and a Y chromosome or an egg with two X chromosomes. Fertilizing a normal (X) egg with this sperm produces an XXY offspring as well as fertilizing a double X egg with a normal sperm also produces an XXY offspring. Second mechanism for preserving the extra chromosome is through a nondisjunction event occour during meiosis II in the egg.[5]

80% of patients have classic form of KS which is 47, XXY karyotype. The remaining 20% have either mosaic 47, XXY/46, XY (i.e. different karyotype in different cells), supernumerary X chromosome aneuploidy (48,XXY; 49,XXXXY), one or several additional Y chromosomes (e.g. 48,XXYY) and cases of 46,XX males have also been reported.[5,6] Sex chromosomal aneuploidies are much less frequent with 48,XXYY and 48,XXXY being present in 1 per 17,000 to 1 per 50,000 male births. The incidence of very rare pattern of KS 49, XXXXY is 1 per 85,000 to 100,000 male births.[7,8]

Symptoms develops in KS are basically signs of testosterone deficiency; very small, firm testes; and infertility. They often display symmetrical gynecomastia with significantly palpable glands and XXY males may have weaker muscles and reduced strength. As they grow older day by day, they become taller than average. They may have less muscle control and coordination than other boys of their age. Some affected individuals also have genital variety including undescended testes (cryptorchidism),the opening of the urethra on the underside of the penis (hypospadias), or notably small penis (micropenis).[3] Approximately 70% of patients complain of falling libido and potency from the age of adulthood and azoospermia is commonly present in more than 90% of cases, while in less than 10% of cases some or all sperm have reduced motility.[2,9]

Suspected KS can be diagnosed as a result of thorough physical examination or of chance observation during treatment of concomitant diseases like thrombosis, metabolic syndrome, osteoporosis, epilepsy, mental retardation, delayed verbal development and language disorders. Testicular volume is measured by ultrasound examination which usually also shows testicular hypoechogenicity. According to WHO guidelines, Semen analysis must be performed to see azoospermia. Laboratory tests can reveal mild anemia as a result of testosterone deficiency. A marked increase in serum Follicular stimulating hormone (FSH) levels is particularly characteristic of KS associated with elevated luteinizing LH values correlate with low testosterone. Testosterone levels, which should always be determined in the morning due to fluctuations during the course of a day, may be either within normal range or abnormally low. Diagnosis is finally confirmed cytogenetically by karyotyping (chromosome analysis on lymphocytes from peripheral blood, or on amniocytes or chorionic villi from prenatal specimens).[10]

To the best our knowledge, no such study on cytogenetic analysis of only Klinefelter syndrome has been carried out in Bangladesh. We conducted the present study to reveal the cytogenetic pattern and clinical features of Klinefelter syndrome patients of Bangladesh.

Methods

This retrospective observational study was conducted in the Cytogenetic Unit of the Department of Immunology at BIRDEM General Hospital, Dhaka for a period of seventeen years from 2000 to 2016. A total of 9,216 patients were included in this study. These patients were referred from different area of Bangladesh for suspected chromosomal abnormality. 336 suspected patients of Klinefelter syndrome was referred by the clinicians of various disciplines, but we also diagnosed KS by karyotyping out of this referral. The suspected chromosomal abnormality includes patients with numerical chromosomal abnormality (e.g. Down's syndrome, Klinefelter

syndrome and Turner's syndrome), ambiguous genitalia, primary amenorrhea (e.g. X-chromosome deletion), adrenogenital syndrome. Among them the suspected Klinefelter syndrome patients were mainly referred with the complaints of lack of secondary sex characteristics, sexual dysfunction after puberty and tall stature, gynaecomastia, small penis and eunuchoid body proportion before puberty. All the patients were subjected to complete genetic examination and pedigree construction was done to exclude non-chromosomal causes of anomaly. Detailed history and physical findings were also noted.

The study followed the conventional peripheral lymphocyte culture by the standard method using the G-banding technique. The protocol employed for karyotyping was as follows: about 2 ml of heparinized blood was collected in a syringe from peripheral veins of the referral patients. Lymphocytes were grown in RPMI (Roswell Park Memorial Institute)-1640, media containing antibiotics (penicillin and streptomycin) and 15% serum supplementation (fetal bovine serum). The phytohaemagglutinin (PHA) was added as a mitotic stimulant and the samples were incubated for 72 hours at 37°C in 5% CO_2 incubator (Forma Scientific, USA). The cells were arrested at metaphase stage of cell cycle with 0.1% colchicine after the incubation. Then after one hour of incubation (with colchicine) the cells were treated with KCL hypotonic solution. After that, the cells were fixed by three times wash with fixative solution (3:1; methanol: glacial acetic acid). All the reagents used were from Sigma Aldrich, Germany. The slides were then stained with Giemsa and air dried. Chromosome analysis was done under 100X magnification. At least 30 metaphase spreads were screened for each patient.

Results

A total of 9,216 suspected patients were analyzed for karyotyping in this observational study and Klinefelter syndrome were detected in 154 (1.67%) patients. The age limit of the patient ranged from 2 years to 51 years. Most of the patients (42.9%) were in the age group of 20–29 years (table – I)

Table I. *Age distribution of patients detected KS in karyotyping*

Age group	Frequency	Percentage (%)
Less than 10 years	4	2.6
10-19 years	38	24.7
20-29 years	66	42.9
30-39 years	42	27.3
40-49 years	2	1.3
More than 50 years	2	1.3

Among the patients diagnosed as KS in karyotyping showed different sex chromosome aneuploidy. Most common was the classical form of KS - 47, XXY (87%) followed by a mosaic form 46, XY / 47, XXY (11%). Rare variety, supernumerary X chromosome aneuploidy such as 47,XXY/48, XXXY, 48, XXXY (figure-2a) and 49,XXXXY (Figure-2b) was also found (Figure 1).

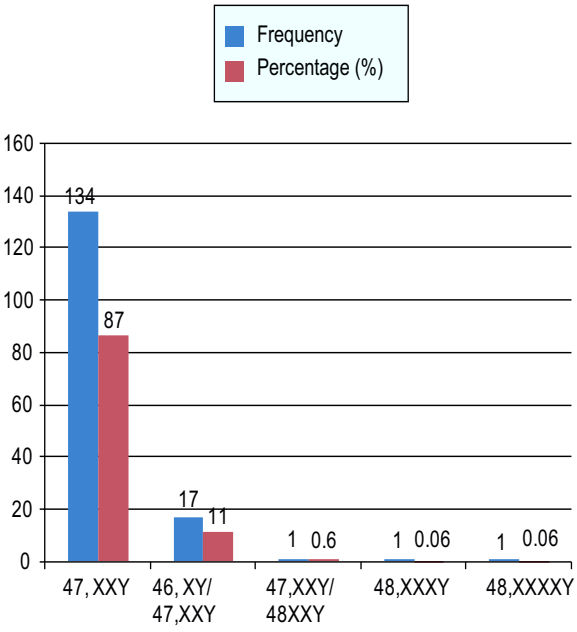

Figure 1. *Genotype variation of KS*

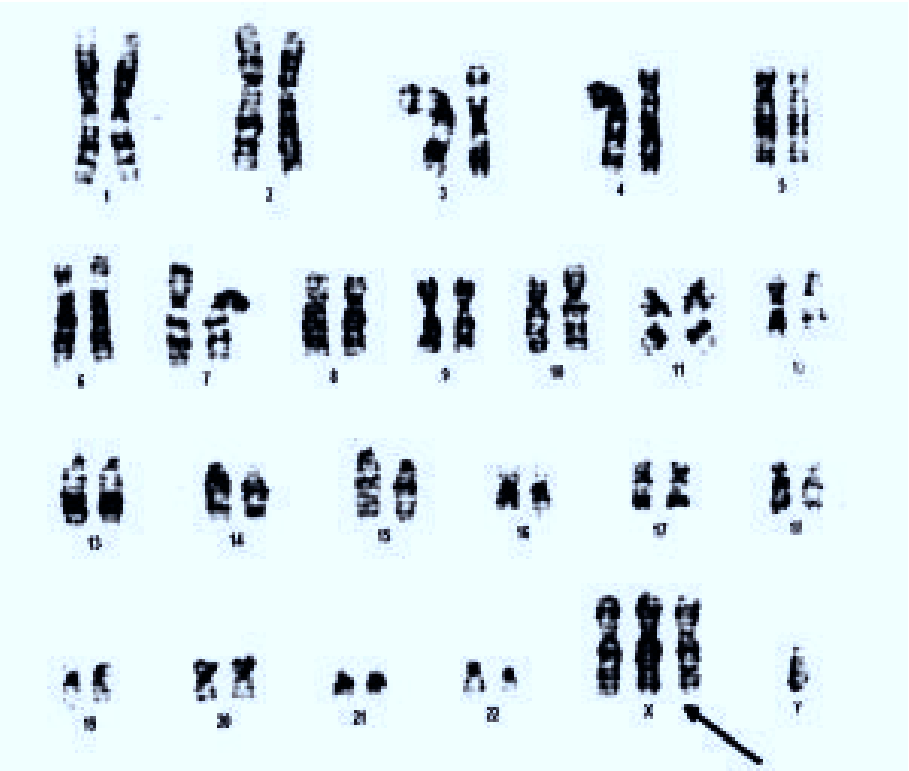

Figure 2(a). *Karyogram showing 48, XXXY karyotpe of an 18-years-old male*

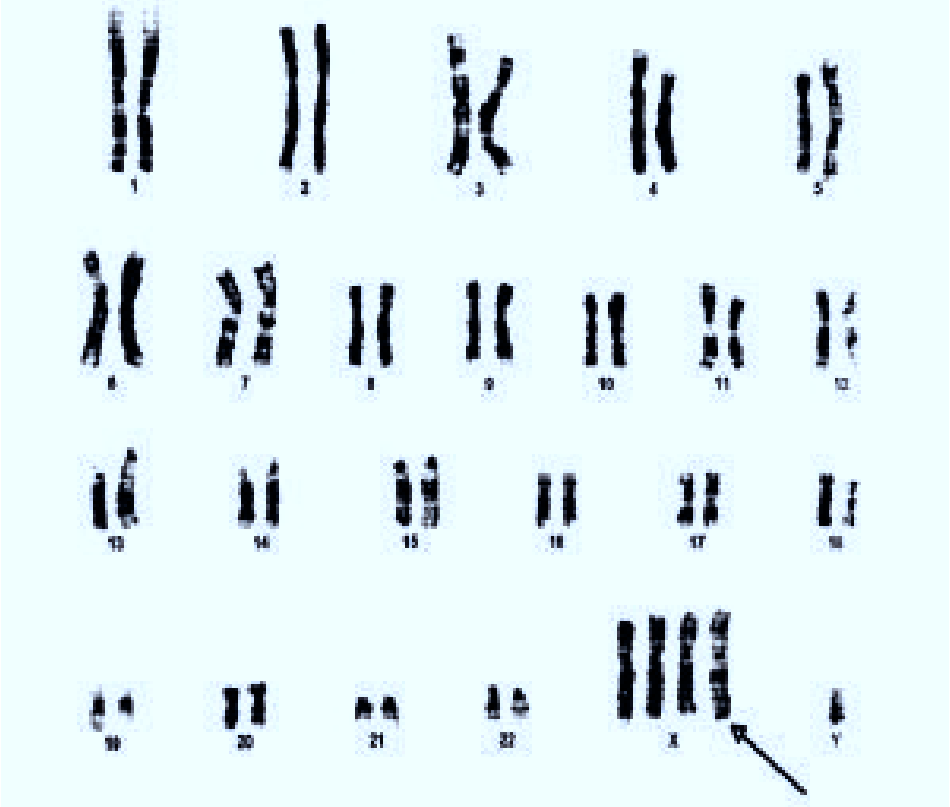

Figure 2(b). *Karyogram showing 49, XXXXY karyotype of a 5-years-old boy.*

Complete physical examination, pedigree construction and semen analysis to diagnose infertility was carried out in all genotypic KS patients. In physical examination 61.7% patients presented with tall stature, 45.5% patients with gynaecomastia, 22.7% patients had small penis, 22.7% patients secondary sex characteristics was not fully developed, Eunuchoid Skeleton found in 29.8% patients and 34.41% patients complained with sexual dysfunction (table - II).

Table II. Phenotypic status in KS

Physical findings	Present		Absent	
	Frequency	%	Frequency	%
Tall stature	95	61.7	59	38.3
Gynaecomastia	70	45.5	84	54.5
Small penis	35	22.7	119	77.3
Lack of secondary sex characteristics	119	77.3	35	22.7
Eunuchoid Skeleton	46	29.8	108	70.2
Sexual dysfunction	53	34.41	101	65.59

In these patient's semen analysis report collected to see azoospermia or fertility status. Azoospermia detected in 20.8% patients and others have normal sperm count (Figure III).

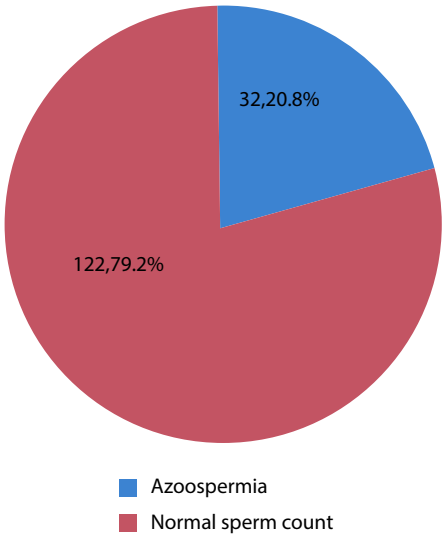

Figure 3. *Semen analysis in KS patients.*

Discussion

In this observational study a total of 9,216 referred patients were analyzed for cytogenetic study by karyotyping and there variations in the frequency of chromosomal aberrations in individuals suspected of having genetic disorders. Among those suspected patients Klinefelter syndrome were detected in 154 (1.67%) patients. Another study carried out in Bangladesh showed that 0.8% patient diagnosed as Klinefelter syndrome.[12] The age limit of the patient rangee from 2 years to 51 years. Most of the patients (42.9%) were in the age group of 20–29 years. Sign and symptoms of Klinefelter syndrome depend on patient's age. In adulthood phenotypic features become more prominent associated with various types of co morbidities. In early age longer lower leg, speech difficulty and small penis are more common than tall stature, gynaecomastia, lack of secondary sex characteristics and sexual dysfunction. In our study clinical suspicion of klinefelter syndrome was 336 referred by the clinicians, but we also diagnosed KS by karyotyping out of this referral.

Approximately one in 1,000 boys is born with an additional X chromosome - 47,XXY, [2] and this sex chromosomal abnormality detected at or before birth in 10 percent of affected boys, and it is found during adulthood in 25 percent of affected men.[11] Almost all men with a 47,XXY karyotype will be infertile. Most of boys with Klinefelter syndrome appear similar to other boys with normal karyotypes. These patients were investigated for sex chromosome study and other hormonal disorders, when infertility or gynecomastia are common presentations. Klinefelter syndrome accounts for 3 percent of male infertility and also common in infertile men with oligospermia or azoospermia (5 to 10 percent).[11] In our study also most of the patients come with fertility problem in the age between 20-29 years.

Most common form of Klinefelter syndrome is 47, XXY karyotype and others have either mosaicism or supernumerary X chromosome aneuploidy. Chromosomal aneuploidies have also been described, although they are much less frequent, with 48,XXYY and 48,XXXY being present in 1 per 17,000 to 1 per 50,000 male births. The incidence of 49, XXXXY is 1 per 85,000 to 100,000 male births. In addition, 46,XX males also be present and it is occurred by translocation

of Y material including sex determining region (SRY) to the X chromosome during paternal meiosis.[4] In our study, Most common was classical form of KS - 47, XXY (87%) followed by a mosaic form 46, XY / 47, XXY (11%). Rare variety, supernumerary X chromosome aneuploidy such as 47, XXY/ 48, XXXY (0.6%), 48, XXXY (0.6%) and 49, XXXXY (0.6%) was also found.

In many cases Klinefelter syndrome remain undiagnosed because of different and robust variation in clinical presentation.[2] Only 25% of patients are diagnosed in their adulthood and few of them diagnosed before puberty.[13] Awareness among general people and professional is about Klinefelter syndrome are few due to lack of adequate knowledge. Early recognition and appropriate hormonal treatment of the disorder can greatly improve quality of life. Lifelong replacement therapy with testosterone supplementation should start at puberty for proper masculine development of sexual characteristics, muscle bulk and bone structure, and to prevent the long-term deleterious consequences without any positive effect on infertility. [13]

Conclusion

Large number of patients remains misdiagnosed due to different phenotypic presentation of Klinefelter syndrome and lack of knowledge among both patients and health professionals. Most of them were diagnosed in their adulthood when they were facing problems such as delayed development of secondary sex characteristics and infertility. But only few of them diagnosed before puberty. To avoid the long term deleterious consequences of the hypogonadism timely testosterone replacement therapy is mandatory. Prompt educational and psychological supports should have to give to prevent difficulties and improve lifestyle. Therefore, we have to focus to improve our ability to diagnose this commonest form of hypogonadism at an early age as well as increase our overall knowledge in genetic diagnosis and related clinical issues.

Conflict of interest: Nothing to declare.

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