

46, XX male: a case study of clinical, hormonal and molecular cytogenetic evaluation of sex development disorder

Naheed Sajjad^{1,2*}, Sarmad Ali¹, Sayedul Haque^{1,3}, Syed Intesar Burney⁴, Sitwat Zehra¹, Syed M. Shahid¹ and Abid Azhar¹

¹The Karachi Institute of Biotechnology and Genetic Engineering (KIBGE),
University of Karachi, Karachi, Pakistan

²Sardar Bahadur Khan Women's University (SBKWU), Quetta, Pakistan

³Human Genetics Program, Department of Animal Sciences, Quaid-i-Azam University, Islamabad, Pakistan

⁴National Research Institute for Fertility Care (NRIFC), Karachi, Pakistan

Abstract: Disorders of sex development (DSD) create medical and social dilemma. Maleness with XX genotype is a rare genetic condition affecting one in 24,000 new-born males. The XX male syndrome is a varied condition characterized by a spectrum of clinical presentation, ranging from normal male genitalia to ambiguous sex. Chromosomal anomalies are important cause of lack of development in secondary sexual characteristics, delayed puberty, miscarriage, infertility and other associated problems. An individual having ambiguous sex may have lifelong impact on social, psychological and sexual functions. The present case study describes the hormonal, clinical and molecular cytogenetics data of sex development disorders in a patient who was phenotypically male but cytogenetic analysis revealed 46,XX.

Keywords: Ambiguous sex, cytogenetics, disorders of sex development (DSD), male infertility, XX male.

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***Author for Correspondence:** naheedsaj@yahoo.com

INTRODUCTION

The XX male syndrome was first described by de La Chapelle and Cols in 1964 and hence is also known as de La Chapelle syndrome¹. Disorders of sex development (DSD) previously called as ambiguous genitalia create complex; medical, social, psychological and sexual problems². Chromosomal anomalies are important causes of lack of development in secondary sexual characteristics, delayed puberty, miscarriage, infertility, etc^{3,4}. Human sexual differentiation is a highly complex phenomena followed by the control of multiple genes and hormones. Abnormalities in normal sexual differentiation are relatively common⁵. However, disorders of sex development is a rare syndrome of sexual differentiation in which testes and male genitalia develop in the absence of Y chromosome and possibly without SRY gene⁶. XX maleness is a varied genetic condition of sex reversal. Maleness with XX genotype is a rare genetic condition affecting one in 24,000 newborn males^{6,7}. In majority of patients, SRY gene is present although SRY negative cases are also reported. About 90% of 46,XX males (testicular DSD) are SRY gene carrier. The remaining 10% cases are SRY-negative. Molecular analysis has demonstrated absence of SRY gene⁸. The phenotypic feature of XX males varies greatly. Some XX males may have normal internal and external male genitals. They may have small testes, abnormal secondary sexual characters and hypospadias. Most males are screened in adulthood due to infertility caused by azoospermia^{5,9-12}.

According to World Health Organization (WHO) infertility is a global health problem¹³. Male infertility is idiopathic in more than 50% of cases^{14,15}. Investigations have described genetic causes which account for 30% of all reported cases¹⁶. Chromosomal abnormalities account for 0.6% for infertility in general population and 2 - 14 % among infertile¹⁷. Human infertility has been closely linked to chromosomal anomalies¹⁸. The present case study describes the clinical details and molecular cytogenetics of sex development disorder in a patient who was phenotypically male but cytogenetic analysis revealed 46,XX.

MATERIALS AND METHODS

Case report

A 20 year old male (II:9) with ambiguous genitalia visited the Reproductive Health Clinic, Jinnah Postgraduate Medical Center (JPMC), Karachi. Proband was a progeny of non-consanguineous parents. He had unremarkable family history having two brothers and two sisters. All brothers and sisters were married, two sisters and one brother had kids (Figure 1).

Clinical assessments

Physical examination and medical history showed height, 144.5cm, weight, 44 Kg, body mass index (BMI) 21.07 Kg/m², gynecomastia, high pitched voice, normal male libido, no complain of reproductive tract infection, and never been surgically operated. Genital examination revealed both male and female type of external genital.

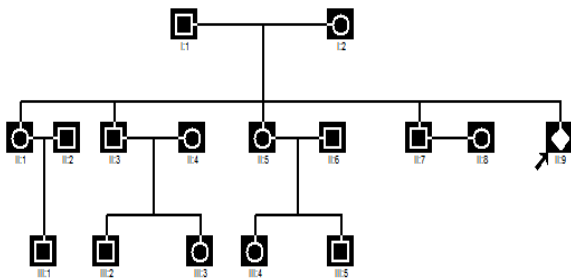


Figure 1: Pedigree of the proband family. Proband II:9 is XX male.

Ultrasound showed normal uterus and ovaries, cervix and vagina were also present, no testes but small penis, adrenal hyperplasia (6x1.5cm) (3x1.9cm). He possessed coarse hair all over his body including auxiliary, pubic and chest area. There was no family history of genetic syndrome, ambiguous genitalia or precocious neonatal death. Patient gave no history of smoking and tobacco addiction. Serum from peripheral blood was used for reproductive hormone investigation.

Cytogenetic analysis

For chromosomal analysis, 3ml of peripheral blood was collected in sodium heparin vacutainer. Blood sample was cultured for 72 hours in RPMI-1640 (Cat no. 11875-119, Invitrogen) with Fetal Bovine Serum (Cat no. 10100-139, Invitrogen), Streptomycin and Penicillin (Cat no. SV30010, HyClone) and Phytohemagglutinin (Cat no. 10576-015, Invitrogen)¹⁹. After harvesting procedure, GTG banding was done. Chromosomal analysis was performed by CytoVision Genus software version 3.93 (Applied Imaging, USA) attached with Olympus microscope (BX 51, USA). More than 30 metaphases were analyzed having good chromosome morphology and minimum overlaps. The karyotype was described according to ISCN 2009 nomenclature²⁰.

Fluorescence In Situ Hybridization (FISH)

FISH was performed using CEP X/Y probe (Vysis, Abbott, USA) on both, interphase and metaphase. Analysis was done using Nikon fluorescent microscope (Eclipse 90i, USA) equipped with FITC green and Texas red filters. Images were captured using CytoVision Genus software version 7 (Applied Imaging, USA).

RESULTS

Hormonal assay

Reproductive hormone profile of the subject is given below (Table 1).

Cytogenetic analysis

Conventional cytogenetic analysis using GTG-banding confirmed the abnormal non-mosaic 46,XX karyotype in more than 30 metaphases (Figure 2).

Table 1: Hormonal assay²¹

S. #	Hormones	Normal Ranges		XX male
		Male	Female	
1	FSH (mIU/ml)	2–15	3–20	1.9
2	LH (mIU/ml)	2–15	<7 (follicular phase) >20 (luteal phase)	13.6
3	TSH (mIU/ml)	0.3–5	0.3–0.5	3.7
4	Estradiol (pg/ml)	15–40	13–20 (follicular phase) 70–250 (luteal phase)	104.4
5	Progesterone (ng/ml)	0.1–1.0	0.64–4.45 (follicular phase) 12.7–79.5 (luteal phase)	10.13
6	Testosterone (ng/ml)	2.4–12	0.1–1.2	13.3

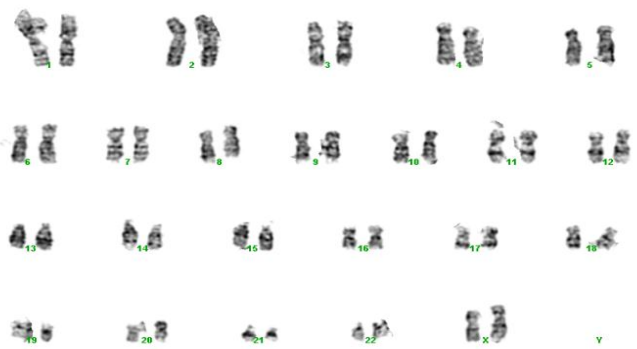


Figure 2: Chromosomal analysis showing 46,XX karyotype.

Two-colour FISH procedure

The presence of XX chromosomes were confirmed by FISH analysis, using fluorescence labeled X and Y centromeric probe (CEP X alpha satellite, Xp11.1-q11.1, spectrum orange; CEP Y satellite III, Yq12, spectrum green). More than 500 nuclei were counted to avoid false interpretation. Both, interphase and metaphase stages were analyzed (Figure 3 and 4). Two red signals showed centromeric region of X chromosomes while no green signal was present. Non-appearance of green signal confirmed the absence of Y chromosome.

DISCUSSION

This case study presents the hormonal, clinical and molecular cytogenetic data of an individual who was phenotypically male. Presence of ovaries, uterus, cervix and vagina were indicating female like genitalia but he did not have any menses. Testes were absent but small atrophied penis, scanty moustache and beard indicate male prototype²²⁻²⁷. The enlarged

adrenal gland was verified by ultrasound which may be due to adrenal hyperplasia. Previous studies have suggested that in most of the DSD patients there is congenital adrenal hyperplasia, which affects the normal genitalia of an individual²⁴⁻²⁷.

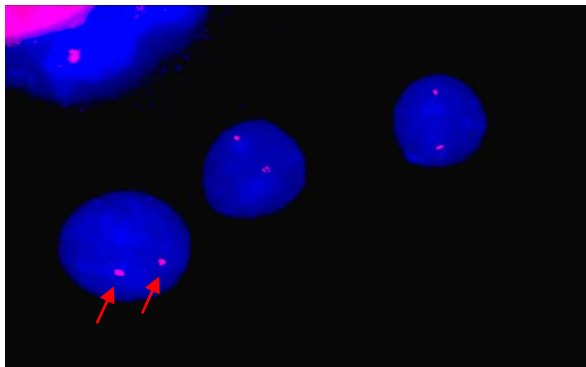


Figure 3: Fluorescent in situ hybridization (FISH) analysis of the X and Y chromosome in interphase cell. CEP X/Y probe was hybridized. Two red signals show XX chromosomes.

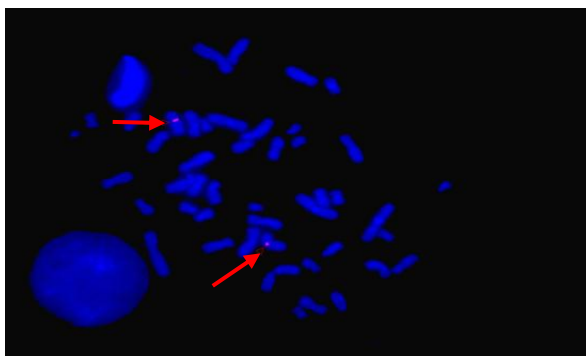


Figure 4: Fluorescent in situ hybridization (FISH) analysis of the X and Y chromosome in metaphase. CEP X/Y probe was hybridized. Two red signals show XX chromosomes.

Hormonal assay showed that follicle stimulating hormone (FSH) and luteinizing hormone (LH) level were towards normal adult male while thyroid stimulating hormone (TSH) level was highly increased than the normal range of both adult male and female. Estradiol level was much higher than normal adult male but it lies within the normal range of luteal phase of normal female. A high progesterone level also coincides with the luteal phase of normal female. Higher level of testosterone than normal female and even that of an adult male was also observed.

Chromosomal analysis showed the presence of two X chromosomes rather than one X and one Y chromosome (figure 2). This result was also confirmed by molecular cytogenetic studies using Fluorescent in situ Hybridization (FISH) using CEP X/Y probe (figure 3, 4). The region Yq12 was

hybridized to check the presence of Y chromosome using above mentioned probe. Non-appearance of green signal for that region showed the absence of either complete Y chromosome or deletion of this particular region.

The sex determining region (SRY gene) on Y chromosome plays important role in the process of sex determination^{28,29}. On the basis of SRY gene studies, 46,XX male patients can be clinically divided into SRY-positive and SRY-negative group^{30,31}. SRY-positive individuals usually have normal male genitalia, small testes, azoospermia and hyper gonadotrophic hypogonadism⁵. Most individuals carry SRY gene on X chromosome during paternal meiosis due to translocation^{5,31,32}. However, in some XX males, SRY autosomal translocations are also reported³³⁻³⁵. The diagnosis of SRY-positive patients is usually done in adulthood during infertility investigations³⁶. The SRY-negative individual includes ovotesticular-DSD, which is characterized by presence of both testicular and ovarian tissues in the gonads of same individual. The diagnosis of SRY-negative testicular-DSD is usually done in childhood upon investigation of ambiguous genitalia^{37,38}. XX males may visit fertility clinics because of the wider application of infertility treatments. However, these patients are not only infertile but sterile too. Their testicular biopsies have revealed a complete lack of spermatogenic cells^{39,40}.

In spite of this fact that chromosomal anomalies are common among infertile men, due to cultural and societal traditions majority of patients deny any clinical or genetic testing as described by Jamal *et al*⁴¹. Focus of this study is to highlight the value of karyotyping of patients having disorders of sex development, as the phenotype does not always correlate with genotype. The identification of such cases is a multi-directional approach involving thorough physical examination, proper clinical investigation and molecular cytogenetic testing to arrive at a precise result. Early and accurate diagnosis of DSD individuals is very important in order to prevent post-surgical, social, psychological and sexual complications.

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