CASE REPORT

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Sex Reversal Syndrome (SRS): A Case of SRY-Positive 46,XX Testicular Disorder

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Abstract

We report a case of an SRY-positive 46,XX Indian male who presented with small testis and phallus, poor beard and mustache development and gynecomastia at the age of 24 years. He was biochemically found to have hypergonadotropic hypogonadism. He had 46,XX karyotype and Quantitative Fluorescence-PCR (QF-PCR) identified the SRY gene on the X chromosome. SRY-positive 46 XX male SRS cases usually present as phenotypically male since birth but develop features of hypogonadism, poor testicular development, and infertility after puberty. Infertility, hypogonadism, external genital development, and psychological distress are the major concerns during the management of the patients. Testosterone therapy for hypogonadism, artificial reproductive technologies for fertility, surgical repair of hypospadias/cryptorchidism/under-virilized genitalia and psychological and genetic counseling are helpful for proper management of the patients.

Key words: Sex Reversal Syndrome, 46,XX with SRY positive, genetic analysis

INTRODUCTION

Sex Reversal Syndrome (SRS) is a form of gender dysplasia that is characterized by an inconsistency between chromosomal and gonadal sexuality. The clinical types include 46,XY female SRS and 46,XX male SRS.[1] A 46,XX male SRS is a rare clinical condition with a reported incidence of 1 in 20000 newborn males worldwide, but there is no exact data in our country.[2] It most commonly occurs due to the translocation of the Y chromosome including the SRY gene on the X chromosome. Sex-determining region Y (SRY) is the major factor for gonadal differentiation.[3] So, the amount of SRY gene present on the X chromosome and the degree of X chromosome activation determines the genital phenotypic variability. In SRY-negative patients, some other genes in the downstream pathway of testicular differentiation like SOX9, SOX3 and RSPO1 are responsible for gonadal differentiation.[2]

Usually, genitalia development is normal and masculine features are obvious in *SRY+* patients except cryptorchidism, small testis and hypospadias.[4] Though testis morphology is normal in infancy, there is gradual hyalinization with azoospermia and reduced testosterone secretion leading to hypergonadotropic hypogonadism and infertility in adulthood.[5] Clinical phenotypes are somehow similar to Klinefelter's syndrome. However, they are differentiated by their short stature, unlike those with Klinefelter's syndrome who are usually tall and pseudo-eunuchoid.[6] Hypergonadotropic hypogonadism, azoospermia on semen analysis, 46,XX karyotyping and genetic analysis for the presence of the SRY gene help diagnose these patients. Fluorescence in-situ hybridization (FISH) and PCR can be used to identify the SRY gene.

Testosterone replacement therapy is the mainstay of treatment for hypogonadism. Equally important are ensuring psychosexual well-being, prevention of osteoporosis and improving quality of life. In subsequent management, fertility issues and psychological and genetic counseling should be addressed.[7]

CASE

A 24-year-old Indian male consulted an endocrinologist for evaluation of small testis and poor beard/mustache development. His medical history revealed that he had been followed for retractile testis on the left side until the age of 8 years. The patient was born at term after an uneventful pregnancy and there was no parental consanguinity. He had a normal libido, good erectile function with normal morning erections and no genital or urinary troubles. He complained of mild asthenia, impaired concentration and breast development in the last 2 years. There was no family history of infertility or other genetic disorders. His Intelligence Quotient (IQ) level was normal.

The physical examination revealed a eunuchoid body habitus (height: 171 cm; weight: 64 kg; arm span: 179 cm; US:LS: 0.85 and a normal male appearance but with scanty

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body, axillary and facial hair. The patient had normal male pubic hair distribution, gynecomastia and normal male external genitalia with no hypospadias and cryptorchidism. Pubic hair Tanner stage was 5 (P5) with a stretched penile length of 10 cm, testicular volume of 6 ml and soft atrophic testis bilaterally. Testicular ultrasonography showed bilateral small testis; the bipolar length of both the right and left testes is about 12 mm. Pelvic ultrasonography showed normal male internal genitalia without any Mullerian derivatives. There was bilateral mild varicocele (Grade l). On semen analysis, the semen sample was translucent and contained no sperm (azoospermia). Hormonal investigations revealed hypergonadotropic hypogonadism (Table 1).

Due to hypergonadotropic hypogonadism, karyotype analysis was performed in two different laboratories revealing 46,XX (Figure 1). For karyotyping, 50 blood lymphocytes were examined at the metaphase stage, where the probability of mosaicism detection was high (around 100%). Subsequently, quantitative fluorescence polymerase chain reaction (QF-PCR) identified the SRY gene on the X chromosome (Figure 2). QF-PCR analysis includes amplification, detection, and analysis of short tandem repeat (STR) markers and non-polymorphic markers. Fluorescently labeled primers are used for the amplification of chromosome-specific markers and, thus, each marker's copy number indicates the copy number of the chromosome. When a chromosome-specific STR marker is heterozygous, two peaks in a 1:1 ratio are found, and when the marker is homozygous, only one peak is observed. The existence of an extra allele as three peaks in a 1:1:1 ratio or two peaks in a 2:1 or 1:2 ratio suggest the presence of an additional STR sequence, which may relate to a different chromosome.[8] A testicular biopsy was proposed to get a histological diagnosis, but the patient refused.

Table 1. Patient's hormone profile

Laboratory Test	Results	Normal values
LH (mIU/ml)	21.60	Adult male: 2.00 - 12.00
FSH (mIU/ml)	22.00	Adult male: 1.00 - 08.00
S. Testosterone (nmol/L)	2.82	20-50 years old (male): 10.40 - 35.71
S. Estradiol (pg/mL)	20.08	Male: 0.00 - 84.00

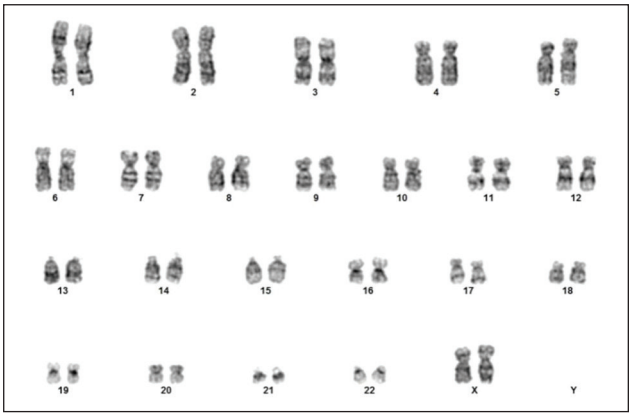

Figure 1. Result of karyotype analysis revealing 46,XX.

Testosterone replacement therapy, in a dose of 250 mg per month, was initiated along with genetic counseling. He was referred to a psychiatrist for psychological counseling.

DISCUSSION

Human sexual development is a complex process determined by different chromosomal and genetic factors and their degree of expression. In the first stage of sexual development, gonadal differentiation is largely determined by the presence of the SRY gene and the subsequent differentiation into genitals. The formation of secondary sex characteristics is dependent on the hormone secreted by the differentiated gonad.[1] Three mechanisms have been postulated for the etiology of 46,XX male DSD: (1) Translocation of Y chromosome including the SRY gene on the X chromosome or on autosomal chromosomes; (2) Secret Y mosaicism found only in the gonads; and (3) X-linked mutation/ overexpression in the genes that cause testis differentiation or mutation/ overexpression in autosomal genes [e.g., SRY box-related gene 9 (SOX9)] in SRY negative XX males.[3] The most common type, translocation of SRY locus containing the Y chromosome on the X chromosome, occurs during paternal meiosis recombination.[2] Due to phenotypic difference, 46,XX males can be clinically divided into the SRY-positive and the SRY-negative groups. SRY-positive 46,XX patients usually present in adulthood with normal-appearing male external genitalia and masculine signs but with small testes and phallus. Genital ambiguity can also be seen in the former, whereas SRY-negative patients usually present with genital ambiguity since birth and varying degrees of masculinization.[2,9] Phenotypes in these patients depend largely, but not only, on the presence of the *SRY*. Other genes like *SOX9*, *SOX3*, *DAX1*, *WT1*, *FGF9*, and *SF1* are also involved in the downregulatory pathway of the sex determination cascade.[6]

Phenotypic variability of SRY-positive 46,XX subjects depends on the amount of Y materials, its position on the X chromosome or the presence of minor deletions or point mutations secondary to the exchange of genetic material and activation of the translocated X chromosome for sufficient expression of the SRY protein.[10] Usually, genitalia development is normal and masculinity signs are obvious in *SRY*+ patients. In the index case genital development, signs of masculinization, erection, ejaculation and sex psychology were also normal but there was a small penis, retractile testis before puberty, gynecomastia, poor beard and mustache development. So, based on phenotypic abnormality, it is difficult to find *SRY*+ male DSD patients before puberty.[4] Like the other 46,XX testicular DSD patients, testis morphology was normal in infancy and there was little increase in testicular size to 6 mL in adulthood. Hyalinization of the seminiferous tubules in early childhood causes loss of spermatogonia. So, the level of testosterone is normal during puberty, but deficiency gradually develops during adulthood leading to hypergonadotropic hypogonadism and infertility.[5] Though SRY-positive 46,XX patients present with hypergonadotropic hypogonadism

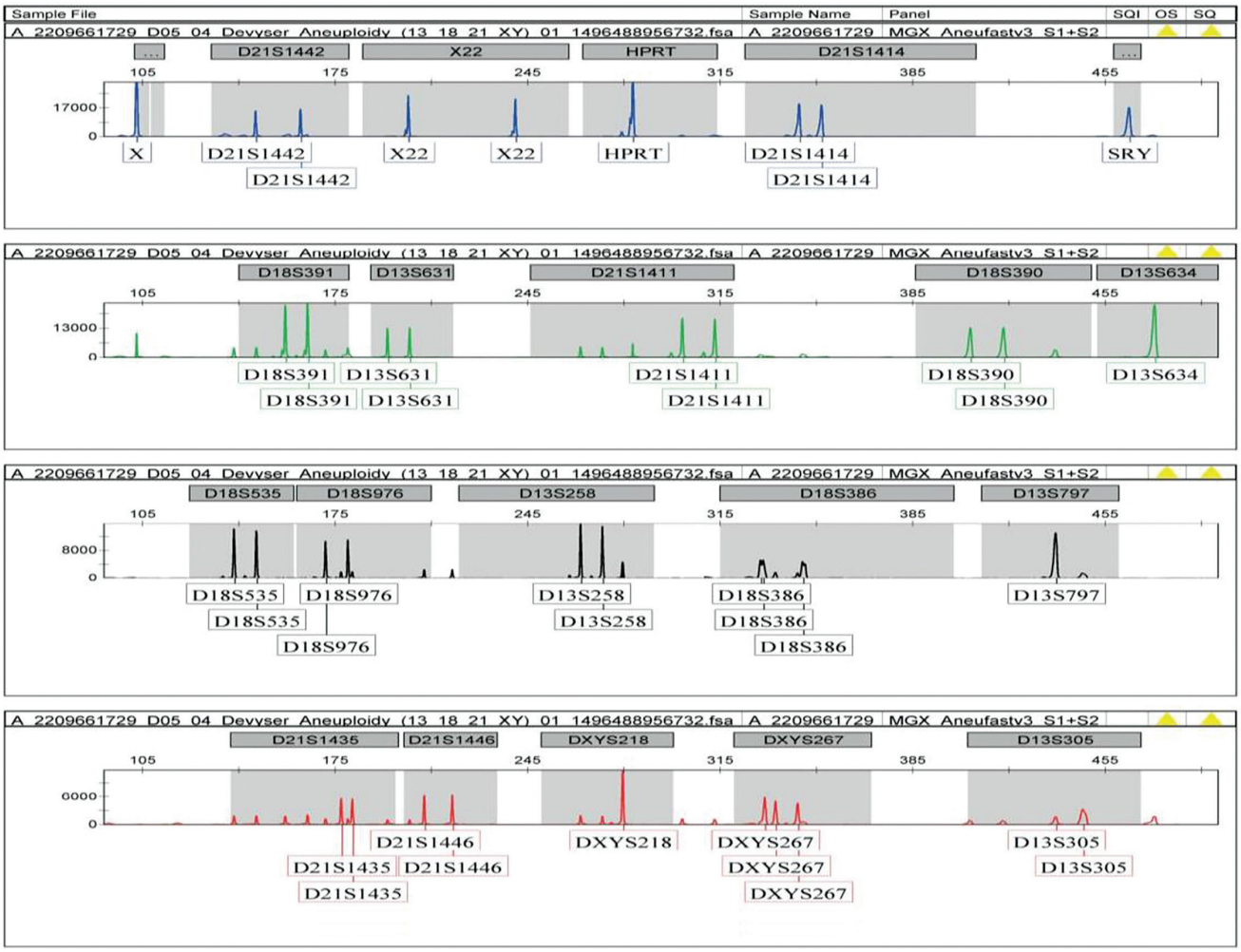

Figure 2. Quantitative fluorescence-PCR (QF-PCR): Upper circle- SRY gene, Lower circle - copies of both X and part of the Y chromosome.

and small testes similar to Klinefelter's syndrome (47,XXY), they are differentiated by short stature, unlike those with Klinefelter's syndrome who are usually tall and pseudo-eunuchoid.[6] In the reported case, there was eunuchoid body habitus which may occur if the hypogonadism develops before puberty.

For diagnosis, appropriate physical examination, trans-abdominal ultrasonography and karyotypic analysis with detection of the SRY gene are required. Semen analysis is mandatory which usually shows azoospermia and so is the karyotype test.[6] FISH and PCR technology can quickly and accurately detect the information about the SRY gene in patients.[1] We identified the SRY gene by QF-PCR method and diagnosed the case as 46,XX male. Phenotypic variability of SRY-positive 46,XX patients, may be explained by the differential inactivation of the X chromosome carrying SRY, thereby influencing SRY expression. X-chromosome inactivation patterns (XCIP) can be assessed by X replication studies, in situ hybridization associated with a modified R-banding technique, or methylation-sensitive PCR analysis of sequences of the androgen receptors (AR) gene, on the active and inactive X chromosomes.[9] So, these techniques help to explain the genotype-phenotype relationship in this group of disorders. These are not available in our country, so the X-chromosome inactivation pattern was not done. During the evaluation of a phenotypically male subject with small firm testis, small phallic length and biochemically hypergonadotropic hypogonadism, karyotyping with detection of the SRY gene is necessary to establish the diagnosis and for the exclusion of other differentials.

Management is based on the presenting signs and symptoms, fertility issues, psychological and genetic counseling and prevention of long-term complications due to hypogonadism. The mainstay of treatment is testosterone replacement therapy to correct hormonal imbalance, prevent gynecomastia and osteopenia/ osteoporosis and maintain normal sexual life as well as improve quality of life. Reduction mammoplasty may be considered in some cases.[7] Psychological support and timely referral to an assisted conception service should be offered. Management options for infertility in couples where the male has 46,XX testicular DSD is artificial reproductive technology including artificial insemination of the female partner with donor sperm. Growth hormone therapy may be considered for short stature.

Surgical repair of orchidopexy and/ or hypospadias, if under-virilized, should be offered. As the patients are phenotypically and psychosexually male, their main psychological distress stems from infertility, sexual dysfunction due to hypogonadism, genotype-phenotype aberration and external genital development (poorly developed to ambiguous).[7] Referral to a mental health professional is, therefore, imperative. Utmost sensitivity is necessary when conveying information about the genetic cause of the disorder and associated sterility. SRY-positive 46,XX testicular DSD is generally not inherited because of infertility of patients and the *de novo* occurrence of Y and X chromosome translocation. The SRY gene, in rare occasions, may be positioned incorrectly on a chromosome other than the X chromosome. This translocation may be carried by an unaffected father and passed on to a child with two X chromosomes, resulting in 46,XX testicular difference of sex development.[10] In SRY-negative cases, the pattern of inheritance depends on the genetic cause, if known. So, during genetic counseling, the mode of inheritance should be addressed.

CONCLUSION

SRY-positive 46,XX males phenotypically present with normal male external genitalia and signs of masculinization except cryptorchidism, small testes, small phallus, and hypospadias. They usually present in adults with small testis, small phallus, hypergonadotropic hypogonadism and azoospermic infertility, similar to Klinefelter's syndrome. For this reason, a phenotypic male with small firm testes, small phallus and hypergonadotropic hypogonadism should have a karyotype done to distinguish from Klinefelter's syndrome (47,XXY) or KS mosaics. If karyotype 46,XX is determined, testing for the presence of the SRY gene is necessary to establish the diagnosis and subsequent management. Infertility, hypogonadism, external genital development, and psychological distress are the major concerns during the management of the patients. Testosterone therapy for hypogonadism, artificial reproductive technologies for fertility, surgical repair of hypospadias/cryptorchidism/under-virilized genitalia and psychological and genetic counseling are helpful for proper management of the patients.

Ethical Considerations
Patient consent was obtained before the submission of the manuscript.

Statement of Authorship
The authors certified fulfillment of ICMJE authorship criteria.

CRediT Author Statement
KS: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data curation, Writing – original draft preparation, Writing – review and editing, Visualization, Project administration; **TF:** Conceptualization, Validation, Writing – review and editing; **TH:** Conceptualization, Validation, Writing – original draft preparation, Writing – review and editing; **MH:** Conceptualization, Methodology, Validation, Investigation, Writing – review and editing, Supervision, Project administration.

Conflict of Interest
The authors have no conflicts of interest to disclose.

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