

Disorders of Sexual Development: Ambiguous genitalia presenting with 45, X/46X, idic(Y) karyotype

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Abstract

*Ambiguous genitalia constitute a rare phenotypic presentation of the urogenital system that can signal an underlying life-threatening disorder. The present case relates to a 3 months old infant reared as male child with the mosaic karyotype 45, X/46, X, idic(Y) who presented with ambiguous genitalia. Genital ambiguity was present in the form of partial fusion of labioscrotal folds and microphallus with single opening at the tip of phallus. The child underwent orchidopexy for right inguinal testis at age of 2 months while left testis was present in labioscrotal sac. Basal testosterone level was 160 ng/dl and LH 3 IU/L. Pelvic ultrasonography did not reveal presence of Mullerian structures. **Material and method:** First Karyotype was performed with peripheral lymphocytes, GTG banded chromosome analysis, the presence and location of SRY gene was investigated using FISH, respectively.*

***Result:** Karyotyping confirms the 45, X (monosomy X), while the FISH analysis revealed presence of two cell lines: one cell line with single copy of X chromosome with absence of SRY gene in 70% of cells. Second cell line with one copy of X chromosome and 2 copies of SRY gene in 30 % cell analyzed.*

***Keywords:** Iso dicentric idic; Mosaicism; orchidopexy; Fluorescence in situ hybridization (FISH)*

Introduction

Normal sex development is a process under genetic control directing the gonads from both parents to either become testis or ovary(Rey 2017a). This differentiation depends upon ability of gonads to produce hormonal factors. Genetic factors in human are responsible for development of sex and gender. Human male and female are basically differentiated by characteristic chromosomal combination that is 46XY constitutes male, and 46 XX constitutes female(Bailez 2017). The change or mutation in genes and hormones associated with sex development leads to ambiguous genitalia. During gonadal and functional differentiation of sex development some genes have been implicated, where suppression of alternate route is achieved by maintenance of somatic sex of gonads(García-Acero et al. 2020). Disorders of sex development are disorders of inherited conditions associated with atypical development at anatomical, chromosomal and gonadal level(Witchel 2018). These are the conditions in which a person is born with reproductive and sexual anatomy that doesn't seem to fit the typical definition of male or female(Disorders of Sex Development - Health Encyclopedia - University of Rochester Medical Center n.d.). These are very rare and have frequency range of 1:4500 that is around 1-3%. DSD are considered as differences of sex development rather than disorders. Earlier, these disorders were known as intersex, hermaphrodite, pseudo hermaphrodite etc. These disorders are generally the deviations from normal sex development(Sanfilippo 2011). These individuals with ambiguous genitalia may be recognised at birth or may present later in life with infertility, virilization and delayed or absent fertility(Miller and Witchel 2014). Congenital adrenal hyperplasia and mixed gonadal dysgenesis are major causes of these disorders. All individuals suspected with these disorders need a thorough diagnostic evaluation. The main aim of diagnosis is to reach specific diagnosis of each child. There must be a structural approach towards DSD, as it will require multidisciplinary team and different molecular techniques(Khanna, Sharma, and Gupta 2019).

There are three stages of sexual differentiation:

- i. Chromosomal sex is determined at conception. It depends on the sex chromosome complement of fertilizing sperm.
- ii. Gonadal differentiation occurs at 6-7 weeks of gestation, and it depends on the SRY gene of Y chromosome. Phenotypic sex is determined at 8-12 weeks of gestation, and it depends upon testosterone and mullerian inhibiting factor.
- iii. Phenotypic sex is determined at 8-12 weeks of gestation, and it depends upon testosterone and mullerian inhibiting factor(Shetty 2018).

Embryology

In humans, sex determination is associated with the sex hormones. Sex chromosome has only one function to perform that is to determine the final morphology of undifferentiated gonad; if Y is present, gonads are testes and if absent then they will be ovaries(Gilbert 2000). About seven weeks of gestation, foetus is sexually undifferentiated with two bipotential gonads and two internally developing wolfian and mullerian ducts(P A and Krishan 2020). These bipotential gonads develop from the urogenital ridge and gets

differentiated into testis or an ovary. If there is absence of testicular tissue functioning, then the female internal mullerian ducts develops (Wilhelm, Palmer, and Koopman 2007). The SRY gene located on the short arm of Y chromosome encodes a factor called TDF (testis determining factor) which guides the testicular development. The differentiation of germ cells present in the urogenital ridge forms two types of cells namely sertoli cells and leydig cells (Harley, Clarkson, and Argentaro 2003). The former is responsible for secretion of MIS (mullerian inhibiting substance) that helps in regression of mullerian ducts while the latter produce testosterone which helps in maturation of spermatogonia and regulates male phenotype. Epididymis, vas deferens, ejaculatory duct and seminal vesicles are all formed from the wolffian duct formed, in the presence of Y chromosome (Sajjad 2010).

- At around 11-13 weeks of gestation the gonads differentiate into ovaries if the Y chromosome is absent. In the absence of MIS, the mullerian structures persist and form fallopian tubes, uterus, cervix and vagina (Rey 2017b).
- Urogenital tubercle, swelling and folds all are included in the undifferentiated external genitalia. In the presence of enzyme 5-alpha reductase, the testosterone present is converted into dihydrotestosterone (DHT) and it leads to the development of these three structures into glans penis, scrotum and penile shaft respectively in males.
- While in the absence of DHT, these structures develop into clitoris, labia majora and labia minora in females.
- The patients with ambiguous genitalia have a different embryology as-
- If the condition is of true hermaphroditism then both testicular and ovarian tissues are present in the gonads.
- If the person has pure gonadal dysgenesis condition then both gonads are streak gonads.
- If the person is present with mixed gonadal dysgenesis then the patient will have testis on one side while streak gonad on the other side.

Epidemiology

The clinical classification in patients is difficult because the phenotypes are similar or almost identical and may have several etiologies (Dreger et al. 2005; Hughes 2007). Due to the lack of clarity of the term, there is no certainty of the incidence of the conditions included. It has been estimated that its individual incidence is approximately 1 in 4,500–5,500 newborns (Sax 2002) and when considering all genital congenital anomalies, including cryptorchidism and hypospadias, the incidence can be from 1:200 to 1:300 (Nordenvall et al. 2014). The incidence of DSD in 46,XY individuals has been estimated in 1 in 20,000 births and the global incidence of DSD in 46,XX individuals (mainly congenital adrenal hyperplasia) is 1 in 14,000–15,000 live births (Pang et al. 1988), which varies by region due to differences in the frequency of pathogenic variants. Congenital adrenal hyperplasia and mixed gonadal dysgenesis constitute half of all patients with DSD which clinically present with genital ambiguity (Thyen et al. 2006).

These conditions can be identified at different times of life, in fetuses or newborns with ambiguous external genitalia, gonadal dysgenesis and internal genitalia that are discordant for the constitution of sexual chromosomes, also can be subsequently diagnosed in individuals with late puberty, unexpected virilization or gynecomastia, infertility or gonadal tumors. Occasionally, DSD may be part of a genetic syndrome, demonstrating the complexity of sexual development and the effect of multiple genes. In recent years, research in DSD has focused on the identification of genetic variants that lead to the atypical development of sex through different techniques. However, sequencing, deletion and duplication analysis have identified causality in near of 50% of cases (Ahmed et al. 2015). It is likely that this diagnostic gap exists due to inadequate knowledge of the pathogenesis and underlying mechanisms of DSD, variation in evaluation and phenotypic description, and limited awareness of the value of molecular genetic diagnosis to guide management and treatment of the individual (Eggers et al. 2016). The challenges facing the genetics of DSD include the development of a diagnostic algorithm that integrates various technologies (including transcriptomics, epigenomics, proteomics and metabolomics), so that the etiology of the entity can be established. This review will discuss basic concepts of DSD and the advances in the diagnostic approach of this entity. Due to chromosomal abnormalities, DSD include Turner Syndrome (45X), Klinefelter Syndrome (47XXY), mixed gonadal dysgenesis or 45X/46XY mosaicism, type of DSD called sex chromosome DSD. There is asymmetric development of gonads in which there is dysgenetic testes on one side and a streak gonad is present on other side. 45X/46XY mosaicism is also associated with short stature, cardiovascular and renal anomalies. On the basis of position and size of missing Y chromosome segment, detection of an abnormal Y chromosome can be done. These are about in the range of frequency 1-3% that is about 1:4500. To determine exact frequency of specific DSDs, data is not available. In different types or forms of DSD like 46XY incidence rate is 1 in 2000 births. For testicular or mixed gonadal dysgenesis-1:10000, for worldwide incidence of 46XX DSD, 1 in 14000-15000 live births. But due to ethnic difference in gene mutation frequency, it also varies with regions.

If we consider, cryptorchidism and hypospadias, which are congenital genital anomalies, the rate will go up as in 1 in 200 to 300. Klinefelter syndrome (1:500 to 1:1000 live births) and Turner syndrome (1 in 2500 live births) are also included.

Case history: The present case relates to a 3 months old infant reared as male child with the mosaic karyotype 45, X/46, X, idic(Y) who presented with ambiguous genitalia (Tuck-Muller et al. 1995). Genital ambiguity was present in the form of partial fusion of labioscrotal folds and microphallus with single opening at the tip of phallus (Hsieh et al. 2002). The child underwent orchidopexy for right inguinal testis at age of 2 months while left testis was present in labioscrotal sac. Basal testosterone level was 160 ng/dl and LH 3 IU/L (McCauley 1990). Pelvic ultrasonography did not reveal presence of Mullerian structures (Aktas et al. 2006).

Methodology

Karyotyping-72-hour stimulated cultures were put up with appropriate mitotic agents. Banding method(s) g-tg-banding with trypsin & giemsa with 450-550 bands pattern (Gartler 2006; Trask 2002).

Fluorescence in situ hybridization (FISH): the SRY region probe is optimized to detect deletions as well as rearrangements of the SRY gene region at yp11(Garimberti and Tosi 2010).

Chromosomal karyotype and FISH analysis

Lymphocytes were obtained at 5 days after birth; 2 ml of peripheral blood was collected, and then 0.5 ml of peripheral blood lymphocytes were cultured in lymphocyte Gibco Roswell Park Memorial Institute (RPMI) 1640 culture medium (Waltham, Massachusetts, United States) at 37°C for 72 h, followed by 50 µg/ml colchicine treatment (Waltham, Massachusetts, United States) 1 h before culture termination to arrest mitoses. The lymphocytes were hypotonically treated in 0.075 M KCl and fixed in methanol:acetic acid (3:1); then G-banding was performed. Immunoassay was performed to detect the infant's serum reproductive hormone levels. Chromosomal analysis of peripheral lymphocytes revealed the presence of cells.

Result

Karyotyping confirms the 45, X (monosomy X) with 30 cell and rest 20 metaphase observed with 46,X,idic(Y) performed on lymphocytes with screening of 50 metaphases revealed 45,X/46,X,idic(Y) mosaicism, while the FISH analysis revealed presence of two cell lines: one cell line with single copy of X chromosome with absence of SRY gene in 70% of cells. Second cell line with one copy of X chromosome and 2 copies of SRY gene in 30% cell analyzed. These findings demonstrate that autosomal retention of SRY can be sub-microscopic and emphasize the importance of karyotyping and FISH in the genetic workup of the monosomic X male.

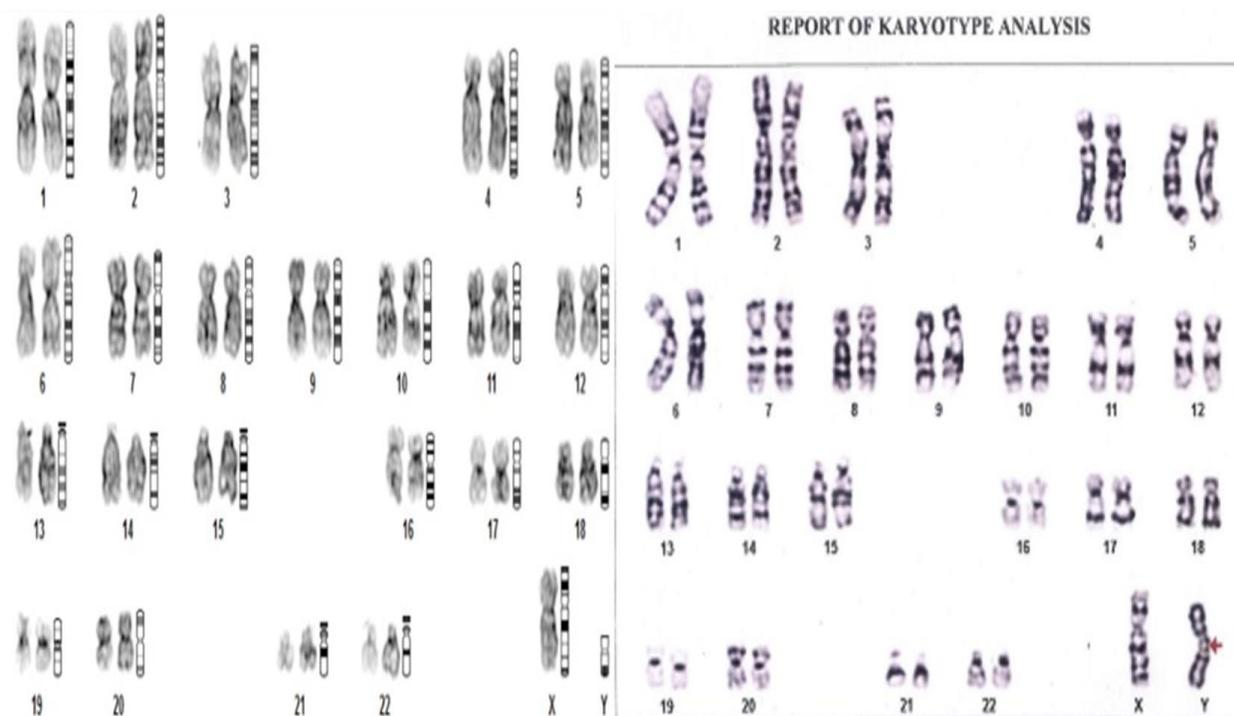


Figure 1: Karyotype counted result image on the left shows mos 45, X and absence of sex chromosome, and image on the right describe 46, X, idic (Y) (p11.3).

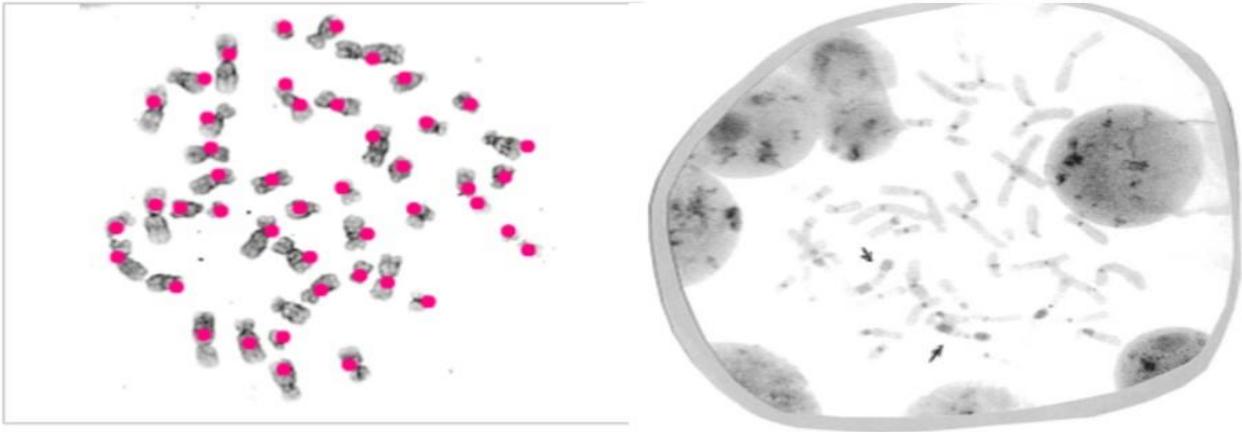


Figure 2: Total counted metaphase arrested cell

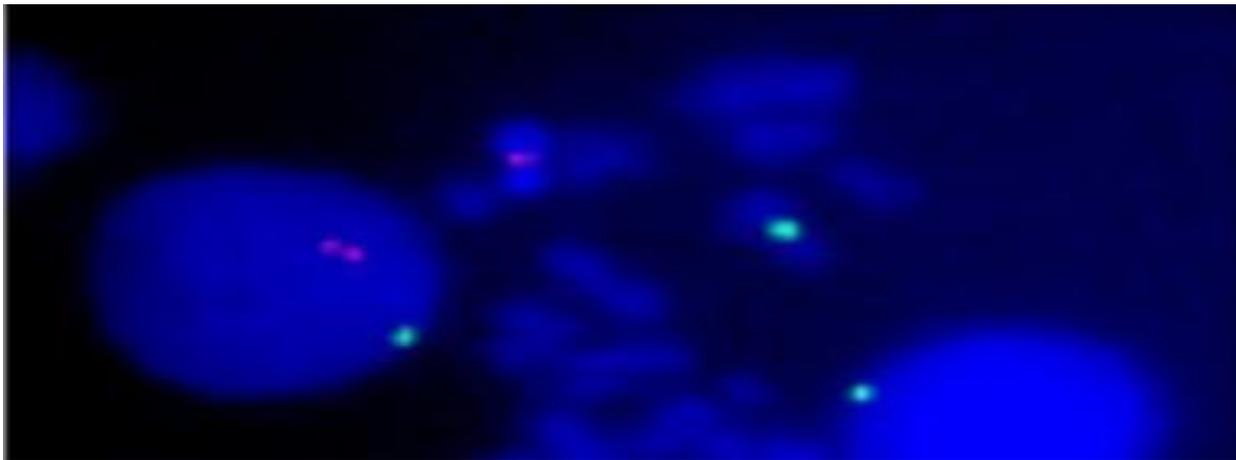


Figure 3: Fish analysis shows signal pattern in left interphase cell showing 2 orange , 1 green signal is indicative of presence of one copy of X chromosome and two copies of SRY gene and signal pattern showing in right interphase cell showing one green signal is indicative of one copy of X chromosome with absence of SRY gene .

Discussion

Anomalies of both number and structure of the sex chromosome lead to alterations in gonadal development(Hashimoto et al. 1997; Henegariu et al. 1997). Manifestations of these alterations range from differentiation of ovo-testis and ambiguous genitalia at birth to gonadal dysgenesis. In this case 45, X/46, Xidic (Y) mosaicism demands careful and through study because its variable clinical feature and its potential complication.

Conflict of interest

Authors have declared that and research was conducted in and the absence of any commercial or financial relationships without any conflict of interest.

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