

Cytogenetic Study on Sexual Ambiguity in Humans

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KEYWORDS Sexual Ambiguity. Intersex. Hermaphroditism. Chromosomal Aberrations

ABSTRACT Sexual ambiguity commonly known as intersexuality, is a condition of imperfect sexual differentiation between male and female. The present study was aimed at analyzing the chromosomal abnormalities of intersex subjects in Coimbatore city, India. Of the 30 intersex cases studied, 10 of them were genetically male (46,XY) but phenotypically females, 8 of them were genetically female (46,XX) with maleness, 4 of them had a 45,X/47,XXY, 6 of them had a 46,XX/46,XY mosaic type and 3 had 46,XY,del(Yq). The results are discussed in the light of recent literature.

INTRODUCTION

Intersexuality is a condition of imperfect sexual differentiation into either male or female. Approximately one in every 4500 live born infants has a genetic anomaly severe enough to make the immediate assignment of sex, difficult. A positive family history, the development of illness suggesting an endocrine/metabolic problem, an impalpable gonad on one or both sides and the presence of other dysmorphic or developmental abnormalities are additional possibilities of an underlying intersexual disorder. Some conditions of intersex disorders result in complete sex reversal, the phenotype being the opposite of what would be detected during childhood when hernias containing gonads are found, or at adolescence when puberty fails to occur. Sexual ambiguity may be due to several disorders of gonadal differentiation, including hermaphroditism, as well as male and female pseudohermaphroditism.

Intersexuality is defined as a "set of medical conditions that feature a congenital anomaly of the reproductive and sexual system, producing embryos and children with sex chromosomes, external genitalia, or internal reproductive system that is not considered standard for either male or female (Intersex Society of North America (ISNA) 2003).

According to Fausto-Sterling et al. (2000), the highest estimates, perhaps 1 percent of live births exhibit some degree of sexual ambiguity, and that

between 0.1% and 0.2% of live births are ambiguous enough to become the subject of specialist medical attention, including surgery to disguise their sexual ambiguity. Other sources (Leonard Sax 2002) create a narrower definition of "true intersexual conditions" and estimate the incidence as far lower, at approximately 0.018%.

True hermaphroditism in humans is an extremely rare condition, the incidence of which has not been determined precisely (Emery and Rimoin 1996). More than 10% of hermaphroditic patients show mosaicism with a 46, XX/46, XY karyotype (Van Niekerk and Retief 1981). Mosaicism following non-disjunction can explain the existence of 46,XX and 46,XY cell lines in a hermaphrodite (Wit et al. 1987).

The varied expressivities of 47,XXY in Klinefelter syndrome result in seminiferous tubule dysgenesis, androgen deficiency, and neurological and cognitive perturbations like language and behavior problems (Simpson et al. 2003).

According to Torres et al. (1996) Hermaphroditism is characterized by the development of ovarian and testicular tissue in the same individual. Mullerian and Wolffian structures are also present.

Before 10 to 15 weeks of development, the fertilized egg of either karyotype has the same internal structures including the gonadal ridges, internal ducts, and external genitalia. The embryo has both female and male sets of internal structures, called the mullerian (female) ducts and wolffian (male) structures. External genitalia at this

point still appear female for both karyotypes (Parker 1998; Kaye et al. 2000).

The genetic sex of the embryo is established at fertilization, the phenotypic sex determining process is set in motion during organogenesis. Apart from sex specific genes present on X and Y chromosomes, autosomal genes also play a role in sex determination (Damiani et al. 1997).

Recent molecular analysis in hermaphroditism revealed that duplication of 22q was recognized in a 46,XX SRY-negative case. A 46,XX hermaphrodite in which SRY was negative in blood leukocytes and epithelial oral cells but present and partially deleted in DNA obtained from the testicular portion of the ovotestes was reported. Also, a study using PCR and FISH analyses revealed the presence of hidden mosaicism for SRY or other Y sequences in some patients with XX true hermaphroditism. Mosaicism for SRY limited to the gonads is an alternative mechanism for testicular development in 46,XX hermaphrodites (Nippon Rinsho 2004).

The conflict of parents and family have also been recognized, because there is a desperate need to classify the infant as male and female surgery and gender assignment have traditionally been based as karyotype and gender identity, feasibility of surgical, cosmetic and functional results and the presence of internal organs – reproductive and oncogenic potential (Reinchenbach et al. 1993). Surgical treatment can include cosmetic procedures to feminize the appearance of the external genitalia when masculinization is marked in genetic females (Schnitzer and Donahoe 2001).

Parents prefer their intersex children to be reared as males, possibly because of the lesser social stigma attached to an impotent male than to a sterile female and because males are socially independent.

With the aim of enlightening the views of the common public concerning hermaphrodites that major scientific phenomenon are responsible for this cause, the present study has been undertaken and this may help the common man to accept this neglected condition and have the way for more care and concern. The present study is designed to analyze the Chromosomal abnormalities of Intersex subjects.

MATERIAL AND METHODS

Individuals of varying ages with sexual ambi-

guity were selected from in and around Coimbatore city. Physically healthy and mentally normal subjects with matching age were treated as controls. Peripheral blood of Cases and Control subjects were collected using heparinised syringe for peripheral blood leucocyte culture following the method of Moorhead et al. (1960) and Hungerford (1978).

RESULTS

All the cases were subjected to peripheral blood leucocyte culture for chromosomal studies. The controls did not display any major chromosomal aberrations except in a case with satellite formation in chromosome 13. Among the patients 9 of them were genetically male (46, XY) but phenotypically had more of female characters. In contrast to the above, 8 of them displayed genetically 46, XX (Fig. 1) complements with physical appearance more of maleness, and 6 of them had a 46, XX/46, XY, where as 45, X/47, XXY

Table 1: Chromosomal complements in the peripheral blood leucocyte culture of sexual ambiguity subjects.

S. No.	Particulars (Case Nos.)	No of metaphase plates screened	Chromosomal complement
1.	4/5/44	50	46,XY
2.	7/8/51	40	46,XX
3.	8/1/49	30	45,X/47,XXY
4.	2/3/53	40	46,XYdel(Yq)
5.	1/7/48	45	46,XY
6.	4/12/75	40	46,XX
7.	8/12/73	30	46,XX/46,XY
8.	28/11/71	35	46,XY
9.	7/9/69	50	45,X/47,XXY
10.	25/3/79	50	46,XX
11.	8/9/79	40	46,XX/46,XY
12.	31/2/68	40	46,XY
13.	27/2/71	45	46,XX
14.	19/7/77	40	46,XX
15.	21/4/69	50	46,XY,del(Yq)
16.	7/11/60	40	45,X/47,XXY
17.	10/1/51	40	46,XY
18.	7/7/59	50	46,XX/46,XY
19.	8/11/48	50	46,XX
20.	20/9/53	45	46,XX/46,XY
21.	7/1/49	40	46,XY
22.	9/11/58	35	45,X/47XXY
23.	11/12/77	35	46,XY,del(Yq)
24.	21/5/78	40	46,XY
25.	5/3/41	40	46,XX/46,XY
26.	2/4/49	50	46,XX
27.	12/7/69	50	46,XX
28.	18/4/66	40	46,XY
29.	19/8/46	45	46,XY
30.	5/5/72	50	46,XX/46XY



Fig. 1. Case No.2/4/49 shows the chromosomal complement of 46, XX

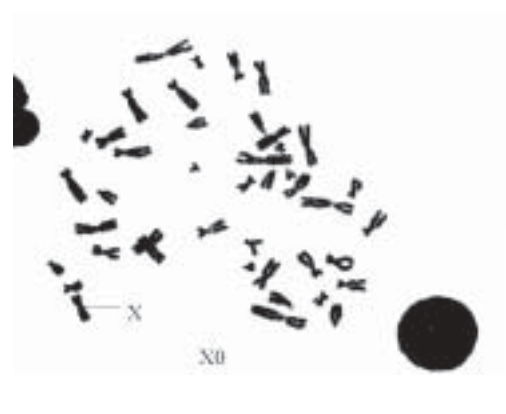


Fig. 2. Case No. 7/9/69 shows the chromosomal complement of 45, XO

(Fig. 2) was found in 4 patients. The remaining 3 subjects showed a deletion in the long arm of Y chromosome 46, XY, del (Yq).

Therefore from the above study, it was observed that 43.3% of the sexual ambiguous subjects showed chromosomal anomalies of various types.

DISCUSSION

Abnormal sexual development may result from multiple etiologies. The differential diagnosis of intersex has long been an interesting intellectual exercise, which has become more fascinating with the unfolding knowledge of genetic and hormonal influence on phenotype development. With proper management it should be possible for all these patients to lead a normal life, although all are sterile.

According to Rey et al. (1999), sexual ambiguity is the expression of low androgen production, the etiology may be either gonadal dysgenesis, resulting in insufficient testosterone and AMH secretion, or isolated Leydig cell dysfunction. Androgen insensitivity is another type of dissociated tubular interstitial testicular dysfunction in which Leydig cell Steroidogenesis is normal, but sertoli cells, which are androgen target cells are insensitive to testosterone, owing to mutation in the androgen receptor.

In the present study, of the 30 patients, 9 of them were genetically males (46, XY) but phenotypically expressed more of female characters.

In contrast to the above, 8 subjects with 46, XX displayed characters towards maleness. 6 of them had a mosaic type of 46,XX/46,XY, and 45,X/47,XXY was found in 4 patients. The rest of the 3 subjects expressed the deletion of long arm of Y chromosome 46, XY, del (Yq). Among controls, one of the Controls expressed satellite formation in chromosome 13.

For all cases, age of the subject is crucial for the decision concerning sex assignment and for an adequate work up, there is a tendency towards the female sex of rearing, as the ovarian tissue is more functional than the testicular tissue, and most patients have mullerian derivatives, and surgical procedures to give the external genitalia a female appearance are more successful.

XX male syndrome is a rare cause of male infertility and literature dealing with it is scanty. In mammals, the sex-determining region of the Y chromosome (SRY) gene is the testes determining factor. The clinical features of male sex reversal syndrome patients are azoospermia combined with one or more of abnormal external genitalia, gynaecomastia, short stature and pelvic cyst. There have been three reports (Zenteno et al. 1997; Kolon et al. 1998; Vernole et al. 2000) describing a total of five male sex reversal syndrome patients with normal male phenotype without SRY gene.

Human males with a 46,XX karyotype are sterile males with normal female chromosomes. The reported incidence varies from 1 in 9000 (de la Chapelle 1972) to 1 in 20000 (Nielsen and

Sillesen 1975) in newborn males. The incidence of 45, X is approximately 1 in 2000 to 1 in 5000 live female births (Frias and Davenport 2003).

The varied expressivities of 47, XXY in Klinefelter's syndrome result in seminiferous tubule dysgenesis, androgen deficiency, neurological and cognitive perturbations like language, behavior problems (Simpson et al. 2003).

In 46, XX patients, ambiguous or male external genitalia reflect androgen action. In a foetus with normal ovaries, the disorder is known as female pseudohermaphroditism and is most frequently caused by congenital adrenal hyperplasia. Alternatively, 46, XX individuals may carry testicular tissue.

Literature describes the three mechanisms to explain 46, XX/46, XY chimerism in patients with ambiguous genitalia.

1. Post – zygotic fusion of two distinct embryos (Green et al. 1994).
2. Fertilization of an ovum and a second polar body by two different spermatozoa and subsequent fusion of the zygotes (Green et al. 1994)
3. Fertilization of a haploid ovum by two different spermatozoa, yielding a 3n zygote, followed by a cell division into three 2nd daughter cells (Plachot and Crozet 1992)

XX males range phenotypically from completely virilized to the hermaphrodites and include a subset of SRY negative subjects. The correlation between genotype (SRY +/-) and phenotype is still unclear (Grigorescu-Sido et al. 2005).

The sex-determining region of the Y chromosome, SRY, is one of the genes required for bipotential gonads to differentiate into testes (Swain and Lovell-Badge 1999). Since the identification of SRY, a number of transcription factors (e.g. DAX-1, SF-1, WT-1, SOX-9, DMRT1/DMRT2) have also been recognized for their role in the differentiation of bipotential gonads into either testes or ovaries (Mendonca et al. 2002; Migeon and Wisniewski 2002). According to Ferguson-Smith (1996) the mechanisms of the co- existence of ovarian and testicular tissues, results of molecular analysis suggest a meiotic paternal exchange between the distal parts of the X and Y chromosome short arms. It can be suggested that the X which has received the Y – translocated material is randomly inactivated. So in cells where the SRY remains silent, the ovary – determining pathway can be switched.

Abnormality of the Y chromosome seems to be frequently associated with 45X / 46XY mosaicism (Telvi et al. 1999; Siffroi et al. 2000; Patsalis et al. 2002; Simoni et al. 2004). A wide phenotypic spectrum has been reported presenting 45X/46XY mosaicism, ranging from females with Turner stigmata and male or female pseudo-hermaphroditism to almost normal male development (Telvi et al. 1999; Huang et al. 2002; Álvarez-Nava et al. 2003).

Intersex disorders can result from errors at any part of the four stages of sexual differentiation. (Parker 1998; Kaye et al. 2000) Fertilization errors, for example, include individuals with Turner Syndrome, who have a 45, XO karyotype, and individuals with Klinefelters Syndrome, who have a 47, XXY karyotype (Woodhouse 2001).

The most appropriate treatment and management of intersex children remains a hot topic of debate, mostly due to the lack of research and knowledge about the long-term medical, functional, cosmetic, psychological, and social outcomes for these individuals. Much of the medical and psycho-evaluative literature comments that intersexuality is a “medical emergency” (Albers et al. 1997; Parker 1998; Kaye et al. 2000; Farkas et al. 2001).

Surgical treatment can include cosmetic procedures to feminize the appearance of the external genitalia when masculinization is marked in genetic females (Schnitzer and Donahoe 2001). One in every hundred people have bodies that differ from the ‘standard’ male or female form, and one or two in every thousand people undergo surgery to ‘normalize’ genital appearance (ISNA 2003).

According to Migeon et al. (2002) the birth of a child with ambiguous genitalia is viewed as a major crisis by most parents. Paediatric endocrinologists, urologists, gynaecologists and psychologists with experience in this field can provide parents with information about normal sex differentiation and the specific abnormality that affects their child. The medical and surgical options should be explained along with our present knowledge of the long-range results of these treatments (i.e. the appearance of the sex organs, sexual function, reproduction, gender).

The studies on hermaphroditism in humans are very scanty, and the exact mechanism leading to sexual ambiguity is still unknown. Further research on both the origins and outcome of intersex conditions promises to greatly the lives of affected people.

The momentous decisions about surgery and sex of rearing of children born with intersex disorders are necessarily made in the pediatric setting, but the consequences are lived out in adult life. It seems obvious that every patient should be given expert medical care and emotional support on a continuous basis from birth, through childhood and adolescence and on into adult life.

In future, due to invasion of scientific advances, the knowledge of the underlying causes of intersex disorders will expand and at the same time, the results of new studies into the long-term psychosexual outcomes of different types of treatment will guide the decision making process.

The challenge will be to discover what makes intersex disorders more acceptable in one culture than in another. Therefore, because of scanty work, this topic needs further an elaborate study from different angles to uplift these neglected subjects of the society. The present study is a step towards future studies on this topic.

ACKNOWLEDGEMENTS

The authors thanks the Physicians of Government Hospital Coimbatore, PSG Hospital Coimbatore, for providing samples and suggestions, and the University Authorities for providing facilities to carry out the study.

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