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Cytogenetic Analysis in Cases with Sex Anomalies

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KEYWORDS Cytogenetic; sex anomalies; mosaic; karyotype

ABSTRACT The relationship between abnormal chromosomal constitution and sex anomalies has been established since the development of cytogenetic methods. Determination of chromosomal status is the prerequisite for precise diagnosis and counseling of any case with sex anomaly. It is also essential for defining genotypic and phenotypic correlation and understanding the basic mechanism involved in sex determination. In the present study, 156 cases with sex anomalies were investigated. 40 cases (25.6%) showed abnormal karyotypes. These included 10 cases with mosacism, of which 7 were with 45,X/46,XX chromosomal complement; 2 had marker chromosome, 45,X/46,X,+mar; and 1 case showed 46,XY/47,XXY constitution.

INTRODUCTION

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). Any alteration in these genes or in the sex chromosomes, leads to abnormalities of sexual development, ranging from complete sex reversal to hermaphroditism. In the present study, results of cytogenetic investigations of 156 cases with sex anomalies are reported.

SUBJECTS AND METHODS

One hundred and fifty six cases with varied abnormalities of sexual development were referred to the Centre for Genetic Disorders during 1991 to 2001, Guru Nanak Dev University, Amritsar, India. Among these, 88 had been raised as females (56.4%) and 68 as males (43.6%). The age of cases reared as females ranged from 26 days to 44 years and those as males from 3 days to 30 years. These cases were referred from different areas of Punjab, Himachal Pradesh, Uttar Pradesh and Bihar. Detailed pedigree analysis and in-depth evaluation of the clinical reports was undertaken in all the subjects. Chromosomal preparations were made from peripheral lymphocytes using RPMI 1640 medium and phytohemagglutinin. G-banding was done as reported earlier (Kaur et al. 1996). In each case 25 to 50 metaphases were examined for numerical as well as structural abnormalities and at least 5 metaphases were karyotyped with Quips (Vysis) Imaging Software.

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RESULTS

Table 1a shows the karyotypic patterns in 88 cases with sex anomalies, that were reared as females. Fifty-five of these females (62.5%) were referred as cases of primary amenorrhea. Six of them (6.8%) had 46,XY constitution. One of them had undescended testes and ovotestes were present in another, while all had hypoplastic uterus (Table 2). Twenty-seven cases (30.7%) had been referred on account of suspected Turner's syndrome, secondary amenorrhea or precocious puberty. Among 6 cases (6.8%) referred as suspected testicular feminization, 46,XY

Table 1a: Karyotypic details of the cases that had been reared as females (n=88)

Referral cause	*Karyotype*	*Number of cases*
Primary amenorrhea	46,XY	6
	45,X/46,XX	3
	45,X	2
	46,XX	42
	45,X/46,X,+mar	2
Turner's syndrome	45,X	1
	45,X/46,XX	3
	46,XX	10
Secondary amenorrhea	46,XX	9
	45,X	1
	45,X/46,XX	1
	46,XY	1
Precocious puberty	46,XX	1
Testicular feminization	46,XX	1
	46,XY	5

constitution was seen in five. Out of 88 females that were studied, only 4 cases (4.5%) showed pure monosomy 45,X; while 7 cases (8%) showed mosaic genotype 45,X/46,XX. In mosaic cases, the ratio of the monosomic cells to the normal cells varied from 5% to 50%. In 6 cases with mosaic cell lines (45,X/46,XX), hypoplastic uterus and scanty growth of axillary and pubic hair were seen while one case was presented with hirsutism. Two cases showed one cell line with marker chromosome (45,X/46,X,+mar).

The second group of 68 subjects, reared as males were referred with conditions like hypogonadism, ambiguous genitalia, hermaphroditism, hypospadias, suspected Klinefelter's syndrome, undescended testes or gynaecomastia. Among the cases referred as suspected Klinefelter's syndrome, 4 (5.9%) had 47,XXY and one (1.5%) showed 46,XY/47,XXY chromosomal constitution. Of 21 cases (30.8%) with ambiguous genitalia, 46,XX karyotype was seen in 4 cases. All 23 cases (33.8%) of hypogonadism had 46,XY chromosomal complement; while among 8 cases (11.8%) of hermaphroditism, 2 cases showed 46,XX karyotype. All 4 cases (5.9%) with undescended testis had 46,XY karyotype; and out of 5 cases of hypospadias, three (4.4%) showed 46,XY, one (1.5%) each had 46,XX and 47,XY,+21 constitution. Amongst 2 cases (2.9%) of gynaecomastia, one (1.5%) had 46,XY,+15q karyotype (Table 1b).

Table 1b: Karyotypic details of the cases that had been reared as males (n=68)

Referral cause	*Karyotype*	*Number of cases*
Hypogonadism	46,XY	23
Ambiguous genitalia	46,XX	4
	46,XY	17
Hermaphrodite	46,XX	2
	46,XY	6
Klinefelter's syndrome	47,XXY	4
	46,XY/47,XXY	1
Hypospadias	46,XX	1
	47,XY,+21	1
	46,XY	3
Undescended testis	46,XY	4
Gynaecomastia	46,XY	1
	46,XY,+15q	1

DISCUSSION

In the present study, out of 88 subjects that had been reared as females, 55 had primary amenorrhea and 11 showed 46,XY karyotype. Five of our cases with 46,XY had underdeveloped secondary sex characters (Table 2). It has been reported that mutations that totally disrupt androgen receptor (AR) function result in complete feminization of a 46, XY individual (Yong et al. 2003). Genetic screening of over 400 such patients (Yong et al. 2000) showed that defects in the AR gene lead to the production of dysfunctional receptor protein in 10% of the cases.

Female phenotype can occur in XY embryo when testis determining factor (TDF) or other genes in the testis determining pathway are lost, mutated or otherwise compromised (Ostrer 2001). Many of such cases become apparent in infancy due to ambiguous genitalia or during adolescence when they fail to have normal puberty.

In our study, pure monosomy 45,X was observed only in 4 cases. Seven of our phenotypic female cases were mosaics (45,X/46,XX) and two cases showed 45,X/46,X,+mar constitution. In one of these cases (case no. 459), Southern analysis of proband and her parents with primers DYZ3 and DYS1, detected Y-specific signals in the proband and her mother confirming that the Y-derived marker chromosome in the proband was of maternal origin (Bashamboo et al. 2003). Amongst 14 cases of Turner's syndrome (TS), one had 45,X and three had 45,X/46,XX constitution. Out of 12 cases of secondary amenorrhea, three cases showed 45,X; 46,XY and 45,X/46,XX constitution, respectively.

The incidence of 45,X is approximately 1 in 2000 to 1 in 5000 live female births (Frias and Davenport 2003). Monosomy X may arise from meiotic non-disjunction or anaphase lagging during spermatogenesis, oogenesis or from postzygotic error. Monosomy for short arm of X leads to short stature and patients usually have the stigmata of Turner (Ferguson-Smith 1965). Some researchers believe that all live born females with Turner's syndrome have a cell line containing two sex chromosomes that may be present at a low level of mosacism (Hook and Warburton 1983; Connor and Laughlin 1989). Nishi et al. (2002) observed that frequency of the Y chromosome sequences in patients with TS varies from 0% to 61% depending on the molecular methodology used and in their cases the most frequent karyotype was 45,X (54%) followed by mosaicism involving structural aberration of X chromosome. Ganguly and Sahni

Table 2: Clinical details of the cases with 46,XY karyotype reared as females

Case no.	*Age (yrs)*	*External genitalia*	*Gonads*
245	26	Female	Ovotestis
246	32	Female (underdeveloped)	Uterine tissue present
462	27	Female	Hypoplastic uterus, ovaries not visualized
511	35	Female (hirsutism)	Hypoplastic uterus, ovaries without follicles
816	37	Female	Hypoplastic bicornuate uterus

Table 3: Clinical details of the cases with 46,XX karyotype reared as males

Case no.	*Referred as*	*Age*	*Gonads/secondary sex characters*
R-49	True hermaphrodite	3.5 months	Common urogenital tract
604	Hypogonadism	22 years	Poorly developed
420	Intersex	3 months	Micropenis, scrotum underdeveloped, no vaginal orifice, uterus present as seen in USG
513	Intersex	15 years	Developed breasts, undescended testis, menarche since14
R-107	Ambiguous genitalia	20 days	Uterus infantile, male type external genitalia, no testis seen in USG
334	Ambiguous genitalia	5 years	Micropenis with urethral opening, testes not palpable, no uterine tissue seen in USG
560	Ambiguous genitalia	4 months	Uterine tubercle behind urinary bladder, two identical swelling
680	Ambiguous genitalia	4 years	Uterine shadow in USG, hypospadias with penile skin, ovary seen on one side

USG: Ultrasonography

(2003) reported that only 1% of the pureline 45,X conceptions are viable, indicating the necessity of mosaicism with X or Y chromosome. Fernandez-Garcia et al. (2000) performed G-banding, FISH, and PCR for SRY in 41 cases of Turner's syndrome and reported 2 cases with Y chromosome mosaicism and 4 cases with 45,X. In 32% of their cases with 45,X/46,XX, the presence of iso-chromosome was seen in 25% and marker chromosomes in 5%. They also reported the presence of Y chromosome and SRY gene in the blood and the ovarian tissue in 2 cases with 45,X/46,XY and 45,X/46,X,idic(Y) chromosomal complement. Abulhasan et al. (1999) reported mosaic cell lines in 21 cases of Turner's syndromes when tested cytogenetically. However, by using FISH probes for X and Y chromosome, a third cell line with 3-7% of cells was detected in 7 cases. Gravholt et al. (2000) examined 114 females with Turner's syndrome for the presence of Y chromosome material by PCR and found that fourteen cases had Y chromosome material. The presence of Y chromosome fragments in patients with Turner's syndrome is known to increase the risk of gonadoblastoma (Mancilla et al. 2003).

In the second group, 68 cases that had been reared as males were referred as having various sex anomalies like ambiguous genitalia, hypogonadism, true hermaphroditism (TH), etc. Three cases referred as hermaphrodites or intersex had 46,XX constitution (Table 3). In 4 cases of ambiguous genitalia and in 1 case of hypospadias 46,XX karyotype was observed while 23 cases of hypogonadism showed normal 46,XY chromosomal complement. True hermaphroditism is an uncommon form of intersexuality in which testicular and ovarian tissue develop in the same individual. Most of the true hermaphrodites are 46,XX and lack SRY. Salas-Cortes et al. (2000) reported that only 15% of 46,XX true hermaphrodites had SRY gene. They found SRY mutations in 15% of cases with 46,XY complete or partial gonadal dysgenesis and in cases of 46,XX/46,XY mosaicism. Okuhara et al.(2000) reported 34 different mutations including 29 missense in the SRY gene in XY females. PCR/FISH studies by Quiepo et al. (2002) revealed hidden mosaicism for SRY and other sequences in some patients with XX true hermaphroditism and showed that mosaicism for SRY was limited to gonads.

Amongst five cases with hypospadias in our series, 46,XY constitution was observed in three, while 47,XY,+21 and 46,XX was seen in one each. Yabumoto et al. (1992) reported sex chromosomal abnormalities in 10 out of 131 cases with hypospadias. In a study on 106 cases with genital

abnormalities, Okten et al. (1998) did not find any chromosomal abnormality in six hypospadias cases.

Out of five cases suspected of Klinefelter's syndrome in our study, four had 47,XXY karyotype while one was mosaic (46,XY/47,XXY). Ozata et al. (1992) in a similar study reported that 24 out of their 29 cases of Klinefelter's syndrome had 46,XY/47,XXY mosaic karyotype. Bojesen et al. (2003) reported a frequency of 40 cases per 100,000 men and found that seven percent of these cases had mosaic karyotype. Advanced maternal age was also found to be a significant contributing factor. Klinefelter syndrome is usually underdiagnosed and less than 10% of cases get diagnosed before puberty. Delay in treatment may lead to decreased muscle and bone mass with subsequent risk of osteoporosis. 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). Studies on mosaic Klinefelter syndromes reveal that the germ cells with sex chromosomal abnormalities were capable of completing meiosis (Mark et al. 1999) and the individuals may reproduce with the aid of modern reproductive technology.

Gender identity is a complex process of differentiation that is affected by numerous variables. The gender assignment in case of intersex individuals is influenced by social, cultural and religious factors and these should be properly comprehended to avoid problems of mismanagement of such cases. Detection of Y-mosaicism in Turner's syndrome is of importance because of the high risk of gonadal tumor development. Timely precise identification and intervention in cases with sex abnormalities is essential for their normal development and proper genetic counseling.

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