

Cytogenetic Investigations in 1843 Referral Cases of Disordered Sexual Development From Andhra Pradesh, India

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KEY WORDS Amenorrhea; infertility; Klinefelter's syndrome; sexual ambiguity; sex chromo-somes; Turner's syndrome.

ABSTRACT Chromosomes play an important role in the etiology of disorders associated with sexual development. A referral case study has been undertaken to know the role of chromosomes in the etiology of these disorders and to establish their frequency. A total of 1843 subjects presenting symptoms of abnormal sexual development referred to Institute of Genetics and Hospital for Genetic Diseases, Osmania University, Hyderabad for cytogenetic confirmation and advice over a period of 20 years (January 1979 to January 1999) formed the subjects for the study. Of the 1843 cases suspected for sex chromosome anomalies, 386 (20.95%) showed abnormal karyotypes. Among these abnormalities, Klinefelter karyotypes were found in 2.88% of the cases and Turnerian karyotypes in 4.12% of the cases. The subjects presenting primary amenorrhea and secondary amenorrhoea had abnormal karyotypes in 11.01% and 0.82% of the cases respectively. In individuals presenting sexual ambiguity abnormal karyotypes were identified in 2.12% of the cases. Chromosome abnormalities accounted significantly for the etiology of aberrant sexual development. It is apparent from the study that cases with history of amenorrhoea, infertility and sexual ambiguity need to undergo cytogenetic evaluation as a mandatory routine. Precise diagnosis of these cases has greatly helped for proper management, surgical intervention and counseling the affected.

INTRODUCTION

Normal sexual development requires the compatibility between genetic sex (X and Y chromosomes), phenotypic sex (body characteristics and external genitalia) and gonadal sex (ovaries and testis) (Forest 1992). Sex chromosomes play a crucial role in the etiology of normal sexual development. The disorders of sexual development associated with aberrant sex chromosome karyotypes are classified into:

(a) *Infertility without Ambiguity*: Klinefelter's syndrome, Turner's syndrome and certain menstrual disorders (Primary amenorrhea and Secondary amenorrhea).

b) *Infertility with Ambiguity in Genital De-*

velopment: True hermaphroditism, mixed gonadal dysgenesis and dysgenetic male pseudohermaphroditism.

c) *Ambiguity without Infertility*: Female pseudohermaphroditism, despite having genital ambiguity are potentially fertile.

Although the etiology is not clearly known, most cases appear to be the result of sporadic errors in meiosis that produce chromosome abnormality by sending a confused or aberrant message to the process of gonadal differentiation.

The occurrence of sex chromosome abnormalities is estimated to be 1 in 448 newborns (Nielsen and Wohler 1991). In a cytogenetic study on 231 females suspected of sex chromosomal abnormality, Anglani et al. (1984) found an overall frequency of abnormal karyotypes in 38.5% cases. In an extensive study on 864 females with genital abnormalities, Chuang et al. (1985) found chromosomal abnormalities in 21.5% cases. In a referral study on 150 cases with complaints of sterility and primary amenorrhea, Joseph and Thomas (1982) reported abnormal karyotypes in 14.7% cases. In a study of abnormal karyotypes in females referred for sex chromosome abnormalities, Ghalib et al. (1988) identified sex chromosome abnormalities in 18.1% of the cases.

So far no referral study reported the combined frequency of sex chromosomal anomalies in disorders of sexual development that include infertility without ambiguity and sexual ambiguity with and without infertility. Therefore, the present study was aimed to establish the role of sex chromosomes and its frequency in the etiology of disorders associated with sexual development. Our's is a twenty year report of cytogenetic evaluation of 1843 referral cases suspected for sex chromosome abnormalities with various disorders of sexual development.

MATERIALS AND METHODS

Over a period of twenty years, from January 1979 to January 1999, a total of 1843 subjects

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presenting the clinical features/symptoms of Klinefelter's syndrome (KF), Turner's syndrome (TS), Primary amenorrhea (PA), Secondary amenorrhea (SA) and Sexual ambiguity were investigated. These cases were referred to the Division of Cytogenetics at the Institute of Genetics and Hospital for Genetic Diseases, Hyderabad, from local and district hospitals of the state of Andhra Pradesh, South India for cytogenetic confirmation and advice. The age of the patients ranged from newborns to 45 years.

All the referral cases were thoroughly examined and detailed clinical and family histories were recorded in special case proformas. The cases were classified according to the reason for referral. 270 males and 1573 females were investigated under the study.

Short term lymphocyte cultures were setup according to the modified method of Moorhead et al. (1960). All chromosome preparations were G-banded according to the method of Seabright (1971). A minimum of 20 metaphases were scored in each case. In cases where mosaicism was detected metaphases upto 50 were analysed and the best among them were microphotographed. Buccal mucosal cells stained with 2% aqueous toluidine blue were scored for X-chromatin (Moore and Barr 1955). Y-chromatin was studied in selected cases according to the method of Borgoanker and Hollander (1971).

RESULTS AND DISCUSSION

The percentage of chromosome abnormalities detected among 1843 referral subjects with disorders of sexual development is presented in table 1. The sex chromosome abnormalities were identified in 386 cases giving a frequency of 20.95%, which includes cases presenting Klinefelter's syndrome (2.88%), Turner's syndrome (4.12%), primary amenorrhea (11.01%),

secondary amenorrhea (0.8%) and sexual ambiguity (2.12%).

Karyological findings in cases presenting infertility without ambiguity (Klinefelter's phenotype and Turnerian phenotype) are presented in table 2a.

Chromosomal analysis plays an important role in determining the cause of Klinefelter's syndrome. It is prevalent in 0.1% in general population (Nielsen and Wohler 1991). In its classical form Klinefelter's syndrome is characterized by tall stature, gynecomastia, testicular atrophy, azoospermia or oligospermia and sterility. Among 118 cases presenting the features of Klinefelter's phenotype, 34 (28.82%) cases showed 47,XXY karyotype, while 19 (16.10%) cases showed 46,XY/47,XXY mosaic chromosome complement. Bockowski et al. (1987) reported a low frequency of 12.5% chromosomal aberrations among cases suspected for Klinefelter's syndrome, whereas a higher frequency of 23.5% was reported by Mitra et al. (1988) in referral cases. Okada et al. (1999) reported a frequency of 7.4% among patients with sterility due to azoospermia. In our study group we observed a very high percent (44.92%) of Klinefelter's karyotype indicating the importance in selection criteria of the suspected cases.

Turner's syndrome afflicts approximately 1 in 2000 females (1998). It is characterized by short stature, webbing of the neck, shield chest, lack of pubertal development, cubitus valgus, ovarian agenesis and primary amenorrhoea. Kim et al. (1999) observed monosomy X in 28.1% cases of Turner's syndrome. In our study 45,XO karyotype was observed in 24.40% of the cases, followed by mosaicism in 17.69% cases.

Table 2b shows the karyotype analysis of cases presenting infertility without ambiguity (primary amenorrhea and secondary amenorrhea). Amenorrhea is the absence of menstruation i.e. either lack of menarche or cessation of menses. Primary amenorrhea is a situation where menstruation has never occurred. It is prevalent in 0.48% of women (Helm et al. 1998). The chromosomal aberrations encountered in their study consisted of X chromosome aneuploidy in 87 (9.21%) cases, mixed and pure gonadal dysgenesis in 17 (1.80%) cases, XY karyotype in 15 (1.59%) cases and the structural changes involving X chromosome in 6 (0.62%) cases. X chromosome mosaics were identified in 74 (7.83%) of the cases. The incidence of chromosomal anomalies in patients with PA has ranged from 20 to 31% (Vaczi et al. 1976; Ten et al. 1990). A study on

Table 1: The percentage of chromosome abnormalities detected among 1843 referral subjects with disorders of sexual development

Type of Disorder	Total number of cases investigated	Number of chromosome abnormalities
Klinefelter's syndrome	118	53 (2.88%)
Turner's syndrome	164	76 (4.12%)
Primary amenorrhoea	944	203 (11.01%)
Secondary amenorrhoea	339	15 (0.82%)
Sexual ambiguity	278	39 (2.12%)
Total	1843	386 (20.95%)

Table 2a: Karyotype results in cases presenting infertility without ambiguity (Klinefelter's syndrome and Turner's syndrome)

Type of disorder	Number of cases	Type of Chromosome abnormality	Number of each type	Total Number of abnormals identified
Klinefelter's Syndrome	118	47,XXY	34 (28.82%)	53
		46,XY/47,XXY	19 (16.10%)	(44.92%)
Turner's Syndrom	164	45,XO	40 (24.40%)	76
		45,XO/46,XX	29 (17.69%)	(46.34%)
		45,XO/46,XX/47,XXX	1 (0.61%)	
		45,X,fragment	2 (1.21%)	
		45,XO/46,X,fragment	1 (0.61%)	
		45,XO/46,XY	1 (0.61%)	
		45,XX t(21;21)	2 (1.21%)	

Table 2b : Karyotype results of cases presenting infertility without ambiguity (Primary amenorrhea and Secondary amenorrhea)

Type of disorder	Total number of cases investigated	Abnormal chromosome constitution	Number and Percent
Primary amenorrhoea	944	45,XO	84 (8.90%)
		45,XO/46,XX	68 (7.20%)
		45,XO/46,XX/47,XXX	2 (0.21%)
		45,XO/47,XXX	2 (0.21%)
		46,XXX/47,XXX	2 (0.21%)
		47,XXX	2 (0.21%)
		48,XXXX	1 (0.10%)
		45,X,fragment	2 (0.21%)
		45,XO/46,X,fragment	1 (0.10%)
		46,XX/46,X,fragment	2 (0.21%)
		45,XO/46,XY	10 (1.06%)
		46,XX/46,XY	6 (0.64%)
		46,XX/47,XXY	1 (0.10%)
		46,XY	15 (1.59%)
		46,XX/47,XX+21	1 (0.10%)
		46,XX/46,Xi(Xq)	1 (0.10%)
		46,XX,13p+	1 (0.10%)
		45,XXt(21;21)	2 (0.21%)
		46,XX	741 (78.50%)
Secondary amenorrhoea	339	45,XO	1 (0.29%)
		45,XO/46,XX	9 (2.66%)
		45,XO/47,XXX	2 (0.58%)
		46,XX/47,XXX	1 (0.29%)
		45,XO/46,XX/47,XXX	1 (0.29%)
		46,XX,13p+	1 (0.29%)
		46,XX	324 (95.58%)

77 patients with PA by Van Niekerk (1978) revealed 27.3% of chromosomal anomalies. Ghalib et al. (1988) and Mulye et al. (1983) revealed a frequency of 18.1% and 22.4% chromosome abnormalities in women experiencing PA. Optiz et al. (1983) and Ten et al. (1990) observed a frequency of 26% and 31% in 88 and 117 women investigated respectively. In our study we observed 21.5% of abnormal karyotypes in women experiencing primary amenorrhea. The present

study based on large sample size (n=944) emphasizes the importance of cytogenetic investigations in women with absence of menstruation after the age of 16 years for better management and counseling.

Secondary amenorrhea is termed when menstruation skips after atleast one menstrual cycle. It is prevalent in 4.9% in women with 4-12 months of amenorrhea duration between 26-35 years of age (Hernandez et al, 1999). The reported fre-

quency of abnormal karyotypes in this group ranged from 0 to 33% (Opitz et al, 1983; Anglani et al, 1984). In the present study, 4.42% (15/339) women presenting secondary amenorrhea showed abnormal karyotypes. Majority of the cases showed X chromosome mosaicism (n=14/15).

Among the types of chromosomal anomalies detected, the frequency of monosomy X condition was more prevalent in Turner's phenotype when compared to amenorrhea. The presence of mosaic condition in cases with Turner's phenotype, primary amenorrhea and secondary amenorrhea reflects the wide spectrum in the clinical picture of these patients, from apparently normal to the Turnerian phenotype. Recent findings of Leonova and Hanson (1999) suggest that most of the mosaic 45,X/46,XX Turner females arise due to the loss of one of the X chromosomes in some cell lines of originally 46,XX conceptuses, rather than through mitotic non-disjunction during early embryogenesis as seen in originally 45,X conceptuses.

Table 3 shows the karyotypic results in cases presenting sexual ambiguity with and without infertility. On the basis of karyotypic results and clinical findings the patients were classified into four groups as True hermaphroditism (TH), Mixed gonadal dysgenesis (MGD), Male pseudohermaphroditism (MPH) and Female pseudohermaphroditism (FPH).

True hermaphroditism was exhibited by 30 subjects who had the chromosomal patterns of 46,XX (11 cases), 46,XY (13 cases), 46,XX/46,XY (4 cases), 46,XX/47,XXY (1 case) and 48,XXXXY (1 case). Mixed gonadal dysgenesis is an abnormality of sexual differentiation, which encompasses a heterogeneous group of different gonadal and phenotypic abnormalities (Alvarez-Nava et al. 1999). The typical characteristic features observed in the present study among

nine subjects with MGD were ambiguous external genitalia, clitoromegaly, hypoplastic uterus and absence of secondary sex characters. Chromosomal analysis revealed 46,XY in 154 subjects presenting MPH. A correlative sex chromatin study of these patients revealed negative for X chromatin and positive for Y-chromatin. Patients with FPH have female internal genitalia and a normal female karyotype (XX) with various degrees of external genitalia virilization (Kamijo and Narita 1997). 85 subjects presenting FPH in our study showed 46,XX karyotype and positivity for X-chromatin.

The observed frequency of 14.02% abnormalities associated with ambiguous genitalia in our study is in accordance with the previous report of Kushch et al. (1990).

Intersex disorders associated with ambiguous genitalia can be caused by different genetic, endocrinological and environmental factors (Forest 1992; Bernard 1970). The birth of a child with genital ambiguity poses both medical and social problems. However, an early intervention would pave a way for better management. This requires a careful physical examination, evaluation of internal and external anatomy and investigations related to genetic component and hormonal assays. The localization of specific genes involved in the process of sexual differentiation has made it possible to determine the mutations and other molecular events and diagnose sexual ambiguity before birth and possibly treat in utero (Wiener 1999).

So far no referral study reported the combined frequency of sex chromosomal anomalies in disorders of sexual development that include infertility without ambiguity and sexual ambiguity with and without infertility. The present study of this nature revealed sex chromosomal abnormalities in 20.95% of the total cases investigated. It is apparent from our data that subjects sus-

Table 3: Karyotype results in cases presenting sexual ambiguity with and without infertility (n=278)

S.No.	Pattern of Ambiguity	Karyotype	Number	Percent (%)	
1.	True hermaphroditism	46,XX	11	3.96	14.02
		46,XY	13	4.68	
		46,XX/46,XY	4	1.44	
		46,XX/46,XXY	1	0.36	
		48,XXXXY	1	0.36	
2.	Mixed gonadal dysgenesis	45,XO/46,XX	6	2.15	14.02
		45,XO/46,XY	2	0.71	
		45,XO/46,X+mar	1	0.36	
		46,XY	154	55.40	
3.	Male pseudohermaphroditism	46,XY	154	55.40	
4.	Female pseudohermaphroditism	46,XX	85	30.58	

pected for Klinefelter's syndrome, Turner's syndrome, amenorrhea and ambiguity have a distinctly higher rate of sex chromosomal anomalies. This data emphasizes the need for the referral of patients with the above criteria for the cytogenetic clinics to establish precise diagnosis that aid for early intervention and better management. The data also demonstrates the indication to establish their frequency and offer proper genetic counseling. The recent advances in prenatal diagnosis and molecular cytogenetics would greatly help in rapid detection of these anomalies.

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