

Chromosomal Abnormalities in 979 Cases of Amenorrhea: A Review

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ABSTRACT Primary amenorrhea refers to absence of spontaneous menarche even after the age of 16 while in secondary amenorrhea, the condition follows a period of normal menstruation. Cytogenetic data in cases with primary (n=852) (PA) or secondary (n=127) amenorrhea (SA) investigated at the Department of Genetics, Dr. A.L. Mudaliar Post Graduate Institute of Basic Medical Sciences, University of Madras, during the 25-year period 1979 to 2004 was reviewed. Routine GTG-band analysis of metaphases from peripheral blood leucocytes revealed the incidence of chromosomal abnormalities in individuals with PA and SA to be 25.82% and 7.09% respectively. In addition to numerical abnormalities, the various structural aberrations of the X chromosome encountered were deletions, isochromosome for the long arm, translocations and ring chromosomes. Ascertainment of the karyotype aided in confirmation of the provisional diagnosis, a better phenotype-genotype correlation to understand clinical heterogeneity and in genetic counseling.

INTRODUCTION

Primary amenorrhea (PA) refers to absence of spontaneous menarche by age 14 years with the absence of growth or development of secondary sexual characteristics or as absence of menses by age 16 years with normal development of secondary sexual characteristics, while in secondary amenorrhea (SA), there are one or more bleeding episodes followed by a minimum of three months of amenorrhea (Doody and Carr 1990).

Genetic causes of amenorrhea account for about 45% of the cases in which ovarian failure due to chromosomal abnormalities include primarily Turner syndrome (TS) and its variants and gonadal dysgenesis. The importance of chromosomal analysis in the evaluation of women with absence of menstruation for better management and counseling has been emphasized by several investigators. The frequency of abnormal karyotypes has been reported to vary between 20 and 31% among women experiencing PA and in up to 33% of women with SA (Opitz et al. 1983; Anglani et al. 1984). In a recent survey on a large sample size (n=944) Jyothy et al. (2002) identified 21.5% of women with PA and 4.42%

women presenting SA to have an abnormal karyotype. The present study was undertaken to determine the cytogenetic profile of patients referred with a complaint of amenorrhea to correlate the karyotype with the phenotypic features.

PATIENTS AND METHODS

A retrospective analysis of the chromosomal anomalies in 981 patients with a complaint of primary (n=854) (PA) or secondary (n=127) amenorrhea (SA), referred for cytogenetic evaluation to the Department of Genetics during the period 1979–2004, was carried out. The patients were referred by clinicians belonging to Government and private hospitals. Data on family history, clinical features and laboratory tests were recorded. A minimum of 25 metaphases (50 metaphases in cases of mosaicism) were analyzed from each individual. Chromosomal anomalies were designated as per standard guidelines.

RESULTS

About 26 percent of the 852 patients with PA

and nine patients (7.09%) diagnosed as SA showed an abnormal karyotype. The type and frequency of aberrations involving the sex chromosomes are detailed in tables 1 and 2. The different structural abnormalities of the X chromosome included deletions of the short or long arm, an isochromosome for the long arm, an isodicentric, ring and X-X or X-autosome translocations. Some of these aberrations are illustrated in Figure 1. The most common abnormal karyotypes observed were 45,X; 45,X/46,XX; 45,X/46,X,i(Xq) and 46,XY.

Turner syndrome manifests itself differently in each female affected by the condition, and no two individuals were found to share the same features in the present study. The common symptoms noted were short stature, broad chest (shield chest) and widely-spaced nipples, streak gonads, amenorrhea, coarctation of the aorta, lymphoedema of the hands and feet and poorly developed secondary sexual characteristics. Turner mosaics showed a wide range of phenotypic variation ranging from Turner to that of normal females. Clinical features seen in XY

Table 1: Constitutional karyotype of patients with primary amenorrhea

S. No.	Cytogenetic category	Karyotype	No. of cases	
			n	%
1	Normal	46,XX	632	74.18
2	Pure Turner	45,X	50	5.87
3	Pure X numerical abnormalities	47,XXX	2	0.23
4	Pure structural X chromosome abnormalities	46,X,i(Xq) 7	30	3.52
		46,X,del(Xp) 5		
		46,X,del(Xq) 10		
		46,X,r(X) 4		
		46,X,idic(X) 1*		
		46,X,mar(X) 1*		
		46,X,Xq+ 1*		
		46,X,t(X;10) 1*		
5	Mosaic Turner with numerical aberrations of X	45,X/46,XX 41	48	5.63
		46,XX/47,XXX 3		
		45,X/47,XXX 2		
		45,X/46,XX/47,XXX 2		
6	Mosaic Turner with structural abnormalities of X	45,X/46,X,i(Xq) 21	30	3.52
		45,X/46,X,del(Xp) 1		
		45,X/46,X,r(X) 6		
		45,X/46,X,idic(X)(q24) 1		
		45,X/46,X,t(X;X) 1*		
7	Pure XY females	46,XY	52	6.1
8	Mosaic XY females	45,X/46,XY 5	8	0.94
		45,X/46,XY/47,XYY/48,YYYY 1		
		46,XX/46,XY 2		
Total			852	99.99

* Shanker et al. (1995)

Table 2: Constitutional karyotype of patients with secondary amenorrhea

S. No.	Cytogenetic category	Karyotype	No. of cases	
			n	%
1	Normal	46,XX	118	92.91
2	Pure Turner	45,X	1	0.79
3	Pure X numerical abnormalities	47,XXX	1	0.79
4	Pure structural X chromosome abnormalities	46,X,del(Xp)	1	0.79
5	Mosaic structural X chromosome abnormalities	45,X/46,X,i(Xq) 3	5	3.94
		45,X/46,X,r(X) 1		
		45,X/46,X,del(Xp) 1		
6	Pure XY females	46,XY	1	0.79
Total			127	100.01

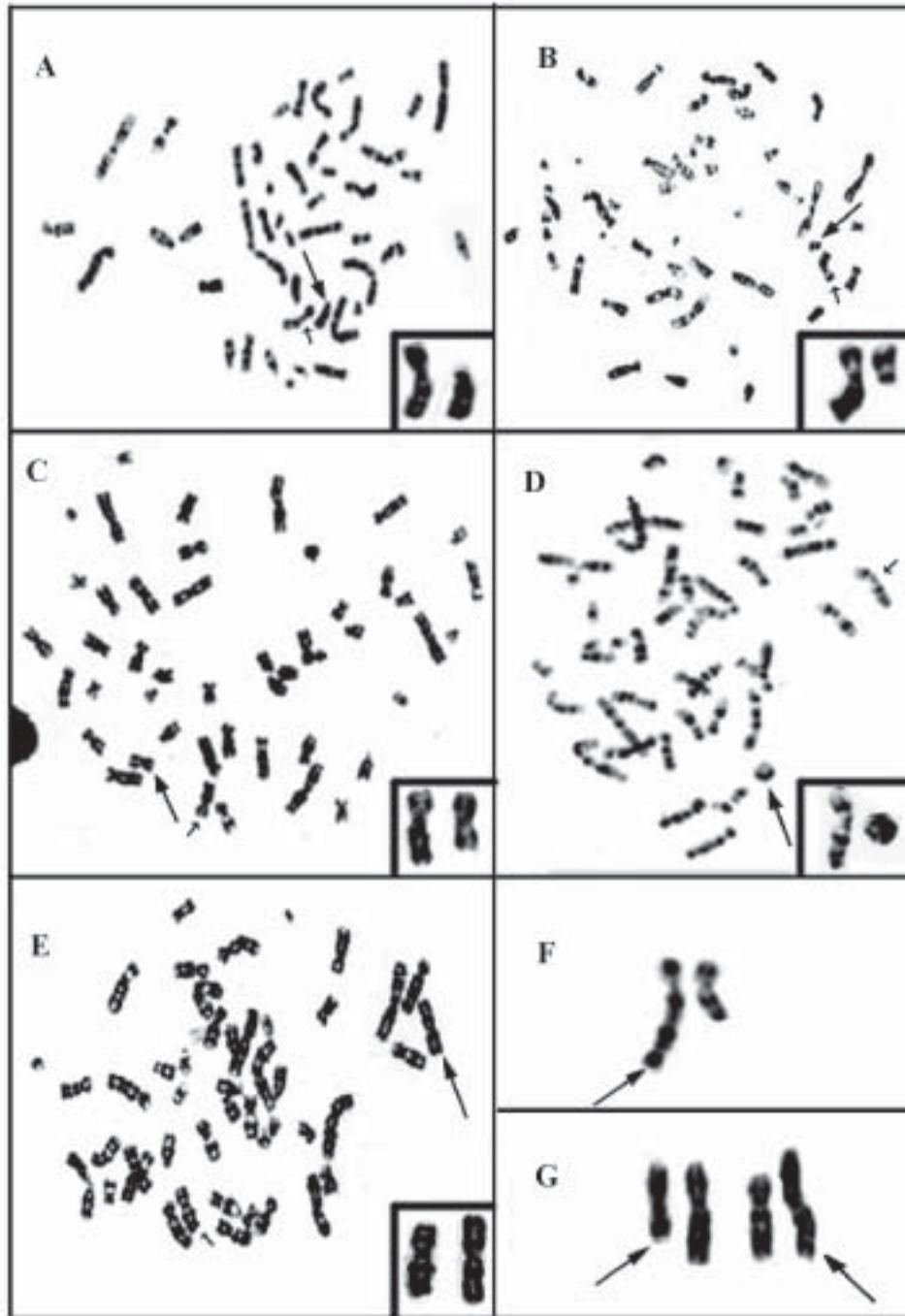


Fig. 1. Structural abnormalities of X chromosome in metaphases from patients with amenorrhea – a) del(Xp), b) del(Xq), c) del(X)(q24), d) ring X, e) i(Xq) [insert shows normal and abnormal X chromosomes]; f and g show partial karyotypes depicting idic(X)(q24) and t(X;10) respectively.

females were mostly a moderately tall stature, hoarseness of voice and absent/hypoplastic uterus and ovaries.

DISCUSSION

A large number of surveys undertaken worldwide to ascertain the frequency of sex chromosomal anomalies in patients present with primary or secondary amenorrhoea have shown a wide variation in the incidence of chromosomal anomalies (Van Niekerk 1978; Joseph and Thomas 1982; Ko et al. 1982; Mulye et al. 1983; Optiz et al. 1983; Chuang et al. 1985; Ghalib et al. 1988; Ten et al. 1990; Park and Kang 1999). A retrospective survey of 620 women with PA and 245 with SA revealed chromosomal abnormalities in 26.13% and 16.33% of the patients respectively (Sayee and Leelavathy 2007). Besides numerical abnormalities of X chromosome, the structural X chromosomal anomalies constituted isochromosome for the long arm, marker, ring and X-autosome translocations. All these aberrations were seen in both pure and mosaic forms. Robertsonian and reciprocal translocations were also documented. A similar frequency of chromosomal abnormalities was also recorded in cases of PA in the present investigation. However, fewer (7.09%) patients with SA exhibited an abnormal karyotype.

Suri et al. (1995) observed 45,X to be the most frequently occurring karyotype (44.4%) followed by 45,X/46,XX mosaicism (24.4%) in their analysis of 45 cases of Turner syndrome. In this study also, 45,X pattern was the most common anomaly noted in 22.7% (n=50) of the cases of PA with an abnormal karyotype. The other common karyotypes identified were 45,X/46,XX in 30 cases (18.6%) and 45,X/46,X,i(Xq) in 21 individuals (9.5%). Wong and Lam (2005) detected sex chromosome anomaly in 24.5% and 9.9% of women with primary and secondary amenorrhoea respectively. In those with PA, male karyotype was identified in 8.4% and X chromosome abnormalities in 16.0%. Fifty-two (23.6%) cases were found to have 46,XY karyotype in this review. It was of interest to note the presence of four cell lines having multiple Y chromosomes in a 45,X/46,XY mosaic female (Table 1).

Detection of chromosomal mosaicism is stated to depend on the number of cells examined, the type of tissue studied and whether *in vivo* or *in vitro* selection against one of the cell lines occurs.

Molecular genetic analysis by PCR amplification and by Southern blot analysis in 91 patients with Turner syndrome and chromosome studies in lymphocyte and fibroblast cultures in 87 of them revealed mosaicism in 58 cases (66.7%). Only 18 (20.7%) were apparently non-mosaic 45,X, and 11 patients (12.6%) showed non-mosaic structural aberrations of the X chromosome (Held et al. 1992). Abulhasan et al. (1999) in their study of 22 cases of Turner syndrome found the same karyotype using both FISH and cytogenetic methods in eight cases (36%) [5 cases of 45,X/46,XX, 2 cases of 45,X/46,X,i(Xq) and one case with 45,X/46,XX/47,XXX]. However, FISH technique identified a third cell line in 7 cases (32%), including 2 cases (9%) with 45,X/46,XY karyotype. Using molecular methods Jacobs et al. (1997) detected two cryptic X mosaics but no cryptic Y mosaics among 211 cases of Turner syndrome. The application of molecular cytogenetic and molecular techniques would aid in the delineation of the genetic etiology in cases of amenorrhea presenting with a normal karyotype. Although there is no "cure" for the adverse impact of chromosomal imbalances on the phenotype (including development), early diagnosis, e.g. Y mosaicism in Turner syndrome, is very important for proper management, prognosis and avoidance of recurrence of chromosome anomalies (Schinzel 2005).

CONCLUSION

This 25-year report of the cytogenetic data on patients with amenorrhea has emphasized the role of chromosomes in the etiology of abnormal sexual development. It has also contributed to the establishment of national database which will lead to a better understanding of the genetic heterogeneity and in turn, to the crucial genes involved.

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