Original Article

Chromosomal Aberrations in Primary Amenorrhea: A Retrospective Study

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Abstract

Objectives: The aim of this study was to estimate the frequency of chromosomal abnormalities and establish the association with clinical of factors such as secondary sexual characters and gonad development in primary amenorrhea (PA). **Study Design:** The study was carried out in a large cohort of PA. The chromosomal aberrations were correlated with secondary sexual characters and anatomical abnormalities. **Materials and Methods:** The data of 490 cases of PA were collected retrospectively. The chromosomal preparations were done from the peripheral blood and subjected to giemsa-trypsin-giemsa banding and karyotyped according to the International System of Human Cytogenetic Nomenclature 2013. The fluorescence *in situ* hybridization was carried out using centromeric and whole painting probes for X and Y chromosome. **Statistical Analysis:** Statistical analysis of the data was performed using online version of social science statistics software. **Results:** A high frequency of abnormal uterus (81.9%) and ovaries (86.7%) were detected in our study. A total of 121 (24.7%) cases were identified with abnormal karyotype. The numerical chromosomal abnormalities were identified in 53 (43.8%) cases while structural abnormalities were identified in 32 (26.4%) cases. The XY karyotype was detected in 29.8% females with PA. The PA individuals with anatomical abnormalities (84.3%) had a high frequency (24.6%) of chromosomal aberrations. **Conclusions:** The present study concluded that cytogenetics plays an important role in precise diagnosis which helps in the management of PA. The cytogenetic analysis should be carried out to know the genetic basis of PA.

Keywords: *Chromosomal abnormalities, karyotype, mosaicism, primary amenorrhea, secondary sexual characters, ultrasonography*

Introduction

Primary amenorrhea (PA) is defined as no menstruation by the age of 14 in the absence of growth or development of secondary sexual characters, as also no menstruation by the age of 16 regardless of the presence of normal growth and development with the appearance of secondary sexual characters.[1] According to World Health Organization studies, 15% of the population are infertile and amenorrhea is the sixth largest major cause of female infertility and 2%–5% of all women affected with PA are in childbearing age.[2]

The normal menstruation is regulated by the pituitary gland which is controlled by hypothalamus. While defining amenorrhea along with menstruation, secondary sexual characters such as pubic and axillary hair and breast development are given due importance. The estrogen is an ovarian hormone which causes duct growth in the breasts resulting in breast enlargement at puberty in girls and is also responsible for the development of other secondary sexual characters. Depending on these anatomical and physiological principles of menstruation, the various etiological factors of amenorrhea can be

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compartmentalized. Overall, it is estimated that endocrine disorders cause PA in approximately 40% of the cases, while the remaining 60% having developmental (genetic or structural) origins.[3] Frequency of chromosomal abnormalities in PA ranges from 16% to 64%.[4] In our study, the types of chromosomal abnormalities were analyzed and correlated with secondary sexual character and anatomical abnormalities.

Materials and Methods

The study was carried out retrospectively on four hundred and ninety (490) individuals with PA referred from 2005 to 2015 to our cytogenetic laboratory. After informed written consent from the patients, the clinical examination was done to determine the provisional diagnosis. All necessary clinical details such as age, height, secondary sexual characters (breast, pubic, and axillary hair development), and radiological details were recorded in the case record sheet. The study protocols were approved by Institutional Ethics Committee.

Chromosomal preparations were obtained from the peripheral blood cultures briefly; 0.5 ml of peripheral blood was added to RPMI 1640 medium (9 ml), supplemented with fetal calf serum (1 ml), L-glutamine (0.1 ml), and antibiotics (penicillin and streptomycin) and stimulated with phytohemagglutinin incubated at 37°C for 72 h; then, the culture was treated with a hypotonic solution (0.075M KCL) and fixed with Carnoy's fixative (Methanol: Acetic acid 3:1).[5] The cell pellets were diluted and dropped on prechilled slides. The chromosomal preparations were subjected to giemsa-trypsin-giemsa (GTG) banding.[6] The chromosomal analysis was carried out from 50 well-spreaded and good-banded metaphases under Nikon 90i microscope, and images were captured with charge-coupled device camera and karyotyped according to International System of Human Cytogenetic Nomenclature 2013.[7] Fluorescence *in situ* hybridization (FISH) was carried out by standard method using centromeric and whole chromosome painting probes for X and Y chromosome (Vysis, Abbott Molecular Inc., Des Plaines, IL, USA).[8] The statistical analysis of the data was performed using online version of social science statistics software (www.socscistatistics.com).

Results

The age of the patients with PA was ranged between 10 and 34 years, and the mean age was 19.20 ± 37 years. The clinical abnormality observed in individuals with PA is presented in Figure 1. A high frequency of abnormal uterus (81.9%) and ovaries (86.7%) were observed in our study. The observed frequency of clinical features including short stature, absence of pubic and axillary hairs, and breast development (<tanner Stage 3) were 45.4%, 66.1%, and 78.5% respectively. The cytogenetic study revealed chromosomal abnormalities in 121 (24.7%) of 490 PA cases [Table 1]. The types of chromosomal abnormality detected in our study were monosomy X, iso (Xq), and dic (X) [Figure 2]. In our series, the frequency of the numerical chromosomal changes (43.8%) was found to be high as compared to the structural aberrations (26.4%). A high (29.8%) incidence of 46, XY karyotype was detected in females with PA. The X chromosome mosaicism was identified in 24 (19.8%) cases of PA.

The correlation of cytogenetic abnormalities with the height of the patients is presented in Table 2. Of 121 chromosomally abnormally PA individuals, 45.4% had

Table 1: Frequency and type of chromosomal abnormalities in primary amenorrhea

Cytogenetic category	Karyotype	*n* (%)
Normal	46, XX	369 (75.3)
Chromosomal abnormality		121 (24.7)
1. Numerical abnormalities		53 (43.8)
a. Pure Turner's syndrome	(45, X)	28 (23.2)
b. Trisomy X	47, XXX	1 (0.8)
Mosaicism of X	-	24 (19.8)
	45, X/46, XX	17 (14.0)
	45, X/46, XY	3 (2.4)
	46, XX/47, XXX	3 (2.4)
	45, X/46, XX/47, XXX	1 (0.8)
2. Structural abnormalities	-	32 (26.4)
a. Deletion Xq	46, X, del (Xq)	10 (8.3)
b. Isochromosome	46, X, iso (Xq)	9 (7.4)
c. Translocation	46, X, t (X; A)	4 (3.3)
d. Idic	46, X, idic (X) p	1 (0.8)
e. Marker chromosome	46, X+marker	4 (3.3)
f. Inversion 9	46, XX, inv (9) 46, XY	4 (3.3)
3. Male karyotype	46, XY	36 (29.8)

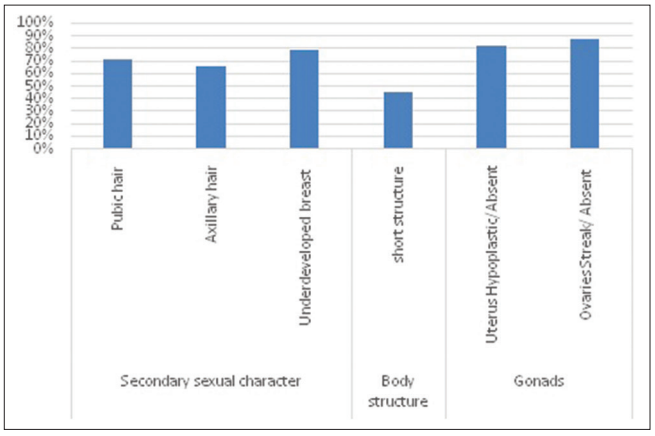

Figure 1: Clinical abnormality frequency in 490 cases with primary amenorrhea

a short stature. A high frequency of short stature was noticed in individuals with monosomy X (89.3%) and structural abnormalities of X (71.9%). The data on secondary sexual characters and anatomical features in PA are presented in Table 3. The pubic and axillary hair was absent or sparse in 66.1% of cytogenetically abnormal cases of PA. A high frequency of the absence of pubic hair or axillary hair was noticed in cases of monosomy X (100%) and structural anomalies (62.5%) of PA cases [Table 3a]. The clinical examination of breast revealed a high frequency (78.5%) of underdeveloped (<3 tanner stage) breast in PA cases [Table 3b]. The ultrasonography examination revealed a significantly high frequency of anatomical anomalies i.e. hypoplastic or absence of uterus (81.9%) and streak or absence of ovaries (86.7%) [Table 4].

Discussion

PA occurs due to several factors including hormonal imbalance, anatomical abnormalities, genetic factors, and environmental factors. Depending on these various etiological factors, amenorrhea can be compartmentalized into those related to the outflow tract (congenital malformation or receptor insensitivity), the ovary (abnormal or absent germ cells and abnormal folliculogenesis), the anterior pituitary (disrupted gonadotropin production or secretion), and the central nervous system (disrupted hypothalamic factor affecting pituitary signaling). However, chromosomal aberrations, especially sex chromosome play an important role in PA. The chromosomal aberration frequency reported to be ranging from 14% to 60% in PA cases.[3,4,9-16] The overall frequency (24.7%) of chromosome aberrations observed in our study is similar to those reported from different parts of the world [Tables 1 and 5]. As one-fourth of PA cases have chromosome aberrations, cytogenetic evaluation is essential for the diagnosis of PA. Our study highlights the importance of the chromosomal analysis in PA cases. In our cohort the correlation of chromosomal abnormalities with clinical presentation suggests that the numerical (80%) and structural chromosome aberrations (70%) influence the clinical presentation (height, secondary sexual characters, and anatomical abnormalities) [Tables 2-4]. Hence, clinical presentation should be considered as indication of sex chromosome involvement in PA. Monosomy X is commonly seen in Turner syndrome. However, all the cases of Turner's syndrome may not be present with classical clinical features. Short stature, poor secondary sexual characters, and anatomical abnormalities along with PA should be considered for the cytogenetic evaluation. In these cases, germ cells, which trigger early puberty and enhance pubertal growth, start to diminish in the end of first trimester of intrauterine life. Consequently only 10%–15% of affected patients could achieve menarche at expected time point.[17-24] The patients who achieved menarche do not maintain it for long time due to gonadal dysgenesis.[25-27] However, time of gonadal dysgenesis vary due to difference in affected region of homologous chromosome.

Some cases of PA remain undiagnosed due to undetected sex chromosome mosaicism. In our study, 24 (19%) females with PA had sex chromosome (45, X/46, XX, 45, X/46, XY, 46, XX/47, XXX) mosaicism [Table 1]. These cases are difficult to diagnose as clinical presentation

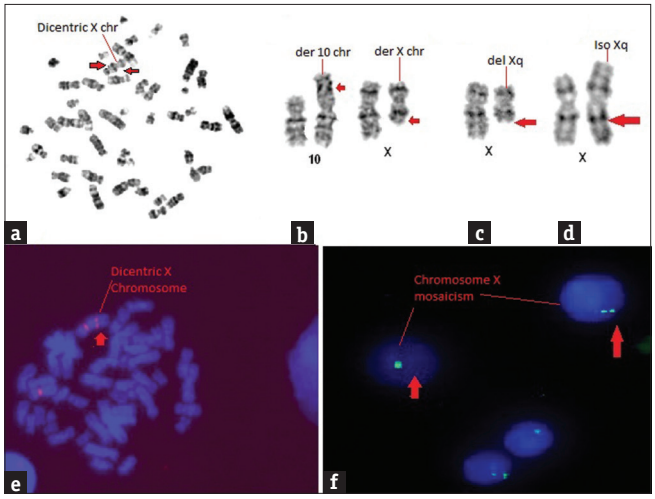

Figure 2: Types of X chromosome abnormalities. (a) Metaphase showing dicentric X chromosome, (b) Partial karyotype of t (10; X), (c) Partial karyotype of del (Xq), (d) Partial karyotype of iso (Xq), (e) FISH showing dicentric X chromosome using centromeric probe, (f) X chromosome mosaicism in interphase cells by fluorescence *in situ* hybridization

Table 2: Association of cytogenetic abnormality and heights of the patient in primary amenorrhea cases

Serial number	Cytogenetic abnormality	Age (years), mean±SD	Height (cm)		*P* (Chi-square test) Level of significance <0.05
			<150 (%)	150 and above (%)	
1	45, X (28)	17.04±3.43	25 (89.3)	3 (10.7)	0.0002 (significant)
2	Mosaicism X (24)	19.71±6.54	7 (29.2)	17 (70.8)	
3	Trisomy X (1)	17±0.00	00	1 (100)	
4	Structural abnormality (32)	19.84±4.57	23 (71.9)	9 (28.1)	
5	Male karyotype (36)	20.28±7.72	00	36 (100)	
Total	121		55 (45.4)	66 (54.6)	

SD=Standard deviation

Table 3a: Association of cytogenetic abnormality and growth of pubic and axillary hair in primary amenorrhea

Serial number	Cytogenetic abnormality	Pubic and axillary hair		
		Present (%)	Absent/sparse (%)	*P* (Chi-square test) Level of significance <0.05
1	45, X (28)	0	28 (100)	0.460 (Insignificant)
2	Mosaicism X (24)	13 (54.2)	11 (45.8)	
3	Trisomy X (1)	0	1 (100)	
4	Structural abnormality (32)	12 (37.5)	20 (62.5)	
5	Male karyotype (36)	16 (44.4)	20 (55.5)	
Total	121	41 (33.9)	80 (66.1)	

Table 3b: Association of cytogenetic abnormality and breast development in primary amenorrhea

Serial number	Cytogenetic abnormality	Breast tanner stage (<3 stage) (%)	Breast tanner stage (>3 stage) (%)	*P* (Chi-square test) Level of significance <0.05
1	45, X (28)	28 (100)	0 (96.4)	0.928 (Insignificant)
2	Mosaicism X (24)	17 (70.9)	7 (29.1)	
3	Trisomy X (1)	00	1 (100)	
4	Structural abnormality (32)	23 (71.8)	9 (28.1)	
5	Male karyotype (36)	27 (75)	9 (25)	
Total	121	95 (78.5)	26 (21.5)	

Table 4: Association of cytogenetic abnormality and anatomical development in primary amenorrhea

Serial number	Cytogenetic abnormality	Uterus			Ovary		
		Normal (%)	Hypoplastic/absent (%)	*P* (Chi-square test) Level of significance <0.05	Normal (%)	Streak/absent (%)	*P* (Chi square test) Level of significance <0.05
1	45, X (28)	1 (3.6)	27 (96.4)	0.009 (significant)	0	28 (100)	0.002 (significant)
2	Mosaicism X (24)	6 (25)	18 (75)		4 (16.7)	20 (83.3)	
3	Trisomy X (1)	0	1 (100)		0	1	
4	Structural abnormality (32)	11 (34.3)	21 (65.6)		11 (34.4)	21 (65.6)	
5	Male karyotype (36)	4 (11.1)	32 (88.9)		1 (2.8)	35 (97.2)	
Total	121	22 (18.1)	99 (81.9)		16 (13.3)	105 (86.7)	

is not indicative for chromosomal analysis. Hence, combination of GTG banding and FISH analysis using X and Y chromosome probes is important to identify undetected mosaicism PA. The cases with sex chromosome mosaicism can be well managed with hormonal therapy as they also had normal cell lineage. Hence, our study strongly suggests the application of FISH investigation in cases of X chromosome mosaicism.

The 46, XY karyotype also has been reported in females with PA. In our cohort, a high frequency (29.8%) of 46, XY karyotype is detected in females with PA which is contradictory to previous published literature from India.[11,13,14,16,28] In our study, the frequency of 46, XY karyotype is high compared to reported literature. Although the reason for the high incidence is not known, the application of FISH could be the one of the factors to detect Y chromosome in PA cases. However, the presence of Y chromosome should be confirmed by molecular cytogenetic technique.[17-19,21-24] At the same time, these cases need to be further assessed for any mutation of SRY and SF1 gene and also in the other genes which are responsible for male karyotype in phenotypic female with PA.[28-32] Gonads developed in such cases do not secrete hormones and should be removed at the time of diagnosis.

Conclusions

The study highlights the importance of cytogenetic investigation in the precise diagnosis and appropriate utilization of FISH in the PA. The X chromosome mosaicism is unnoticed in females with PA as they are phenotypically normal. The interphase FISH should be carried out to rule out the mosaicism. As the genetic factors are limited to chromosome abnormalities, the copy number variations may be playing a role in PA.

Table 5: Chromosomal aberration frequency reported from various studies

Zone	Study	Authors	Number of cases	Percentage abnormality reported
South Asia	India	Roy and Banerjee[11]	60	38 (63.5)
		Lakshimi Kalpana and Satyanarayana[13]	70	20 (28.57)
		Mondal *et al*.[14]	72	24 (33.34)
		Rajangam and Nanjappa[3]	620	162 (26.13)
		Hariharan *et al*.[16]	51	26 (50.8)
		Vijayalakshmi *et al*.[17]	140	39 (27.85)
		Merin *et al*.[18]	246	36 (14.64)
		Amin SV *et al*.[19]	98	20 (20.5)
		Ghosh *et al*.[20]	150	36 (24)
	Malaysia	Ten *et al*.[10]	117	36 (30.8)
	Pakistan	Rizwan and Abbasi[21]	19	5 (26.32)
	Thailand	Tanmahasamut *et al*.[22]	295	59 (20)
Western Asia	Turkey	Temoçin *et al*.[12]	68	18 (26.5)
	Iran	Safaei *et al*.[23]	220	44 (20)
East Asia	Honkong, PRC	Wong and Lam[4]	237	58 (24.5)
South Africa	South Africa	van Niekerk *et al*.[9]	77	21 (21.7)
	Egypt	El-Dahtory[24]	223	46 (20.63)
North America	Mexico	Cortés-Gutiérrez *et al*.[15]	187	78 (41.72)

These variations need to be correlated with clinical abnormalities for better understanding of genetic basis of PA. This will also help PA patient in marital counseling and the future family planning.

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Conflicts of interest

There are no conflicts of interest.

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