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ORIGINAL ARTICLE

Specific Chromosomal Aberrations in Primary Amenorrhoea: Study on 3776 Cases from Indian Population

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Abstract

Objective To verify the prevalence of chromosomal abnormalities in women with primary amenorrhoea in India aiming at appropriate genetic counselling.

Methods In a 16-year retrospective (2001–2016) study, 3776 women with primary amenorrhoea were evaluated. Chromosomal analysis of all the cases was done by GTG banding. Clinical history and other laboratory findings were taken into consideration to determine the diagnosis.

Results The karyotype results revealed 31.2% cases with chromosomal abnormalities ($n = 1177/3776$). In patients with abnormal chromosome complement, 31.2% exhibited numerical aberrations ($n = 367$) and 34.9% with structural aberrations ($n = 411$). About 33.9% of cases were with XY male karyotype ($n = 399$).

Conclusion As per the literature till date, this study is the largest with high incidence of chromosomal abnormalities; early detection of abnormalities is necessary for guidance to reproductive management and genetic counselling.

Keywords Primary amenorrhoea · Mosaicism · Autosome–autosome translocations · Balanced translocations · Genetic counselling

Introduction

Amenorrhoea (absence of menses) can be a transient, intermittent or permanent condition resulting from dysfunction of the hypothalamus, pituitary, ovaries, uterus or vagina. It is classified as either primary (absence of menarche by age 16) or secondary (absence of menses for more than three cycle intervals or 6 months in women who were previously menstruating).

Primary amenorrhoea (PA) can be diagnosed if a patient has normal growth and secondary sexual characteristics but no menarche by 16 years of age. If a patient has no secondary sexual characteristics and no menarche, primary amenorrhoea can be diagnosed as early as 14 years of age [1]. The differential diagnosis of amenorrhoea is broad and can range from genetic abnormalities to endocrine disorders and psychological, environmental and structural anomalies.

Amenorrhoea is a normal feature in prepubertal, pregnant and postmenopausal females, and it accounts for 20% of patients with infertility. It is a clinical symptom that may result from various causes and occurs in 1–3% of women in the reproductive age [2]. Disturbances in structural and functional integrity of female genital tract, ovaries, pituitary gland and hypothalamus or abnormalities in the chromosome constitution may result in PA. Chromosomal abnormalities are considered to be the second most common cause of PA [3]. Majority of the chromosomal aberrations are due to Turner syndrome and its variants (such as monosomy X, deletions of the X chromosome, isochromosome X and ring chromosome X). It has been reported that the percentage of chromosomal abnormalities varies from 15.9 to 63.3% in patients with primary amenorrhoea [4].

Since the psychological and social impact is high, patients seeking genetic evaluation are a small fraction of affected

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women, and hence, the exact incidence or prevalence of PA is less known in India, though studies from south India have reported the genetic basis of PA [4–7]. In view of the above, the present cytogenetic evaluation was undertaken to determine the frequency of chromosomal abnormalities in subjects of primary amenorrhoea referred by clinicians from various hospitals across India.

Materials and Methods

Retrospective studies of 3776 subjects with primary amenorrhoea were referred to our clinical reference laboratory from all parts of India for chromosome analysis during 2001–2016 period. A complete clinical assessment and information pertaining to age, birth order, height, etc., were recorded in a special case pro forma prepared by the clinicians. The institutional review committee of the SRL Limited, Mumbai, India, approved the study. Written consent was obtained from the parents of the subjects; where the parents were not available, consent was obtained from their siblings to perform the test. For all the samples, unique identification number was given before starting the process of sample. The age of the subjects ranged from 14 to 28 years.

Cytogenetic analysis of peripheral blood lymphocyte culture was performed using the modified standard protocol of Moorhead et al. [8]. Each patient sample was analysed for 20 metaphases or more than that at 500-band resolution level by a cytogeneticist. The images of five or more well-spread metaphases for each case were captured and karyotyped using Ikaros Metasystem Software (Altlussheim, Germany). In case of mosaicism and translocation, 50 metaphases were analysed to confirm the abnormality. The standard nomenclature was designated according to the ISCN 2016 [9].

Results

A total of 3776 primary amenorrhoea cases were referred for conventional cytogenetic analysis with a median age of 21 years (14–28 years). The most common clinical findings in primary amenorrhoea subjects were poorly developed or absence of secondary sexual characteristics, absence of uterus and ovaries, short stature, webbing of neck, cubitus vulgaris, hypergonadotropic hypogonadism and androgen insensitivity syndrome.

Chromosomal analysis revealed 31.2% ($n=1177$ of 3776) with abnormal chromosomal complement and 68.8% ($n=2599$ of 3776) with normal chromosomal constitution. The result of the chromosome analysis is given in Table 1. In subjects with abnormal chromosome complement, numerical aberrations were found in 31.2% ($n=367$ of 1177) and structural aberrations in 34.9% ($n=411$ of 1177) subjects. A total of 399 cases (33.9%) were found to have XY female genotype.

Of 1177 abnormal cases, a total of 31.2% subjects who had ($n=367$) numerical aberrations were all Turner variants where 23.0% ($n=271/1177$) subjects exhibited complete monosomy of 45, X with Turner stigmata, 5.4% ($n=63/1177$) with Turner mosaic pattern of 45, X/46, XX; about 2.8% ($n=33/1177$) subjects revealed mosaicism with triple X cell line. Most of these subjects presented with history of cubitus vulgaris and POI (primary ovarian insufficiency).

Structural abnormalities were observed in 411/1177 (34.9%) cases. Among them, the most common aberrations were isochromosome for the long arm of X (Xq) and short arm of X(Xp) found in 16.3% ($n=192/1177$) subjects, followed by deletions of either short or long arm of chromosome X in 7.2% ($n=85/1177$).

Chromosomal translocations were observed in 3.6% ($n=42/1177$). X-autosome translocations were found in 1.9% (23/1177) and autosome–autosome translocations in 1.6% (19/1177) subjects. X chromosome structural abnormalities which included mosaicism with marker chromosome, isodicentric X chromosome and ring chromosome X were observed in 1.9% ($n=22/1177$), 1.9% ($n=22/1177$) and 1.8% ($n=21/1177$) subjects respectively. Other structural abnormalities like inversions, duplications, add(Y), insertion (5; 9), add(X), idic(Y), inv(11) were observed in 2.3% ($n=27/1177$) in the current study. Polymorphic and heteromorphic variants were observed but not included in this study.

Discussion

In the general population, amenorrhoea was detected in 2–5% women of child-bearing age [4]. Genetic factor has an important role in amenorrhoea which accounts for approximately 45% cases as a result of gonadal dysgenesis, chromosomal disorders or Müllerian agenesis [6].

In the present study, the age group of the subjects ranged from 14 to 28 years, mean age being 21 years. The late presentation of PA cases for cytogenetic studies has been reported from Sri Lanka where mean age was 20.5 ± 5.1 years [3].

Worldwide a large number of surveys were undertaken to ascertain the frequency and kind of chromosomal abnormalities in subjects with primary amenorrhoea where a variation in the incidence of chromosomal anomalies was observed [2, 3, 7, 10] (Table 2).

In the present study, a total of 3776 cases were studied, which is the largest till date. It revealed 31.2% ($n=1177$ of 3776) chromosomal abnormalities, which is in accordance

Table 1 Chromosomal analysis of patients with primary amenorrhoea ($n=3776$)

Type of abnormality	Karyotype		Total no. of cases (%)
Normal karyotype	46, XX		2599 (68.8%)
Abnormal karyotype			1177 (31.2%)
		Percentage out of total number of cases ($n=3776$)	Percentage out of abnormal cases ($n=1177$)
Numerical abnormality		367/3776 (9.7%)	367/1177 (31.2%)
Pure monosomy X/turner syndrome	45, X	271/3776 (7.2%)	271/1177 (23.02%)
Turner mosaic	mos 45, X/46, XX	63/3776 (1.7%)	63/1177 (5.4%)
Monosomy X and triple X mosaic	mos 45, X/47, XXX mos 45, X/47, XXX/46, XX mos 47, XXX/46, XX	33/3776 (0.9%)	33/1177 (2.8%)
XY female	46, XY mos 45, X/46, XY/XYY	399/3776 (10.6%)	399/1177 (33.9%)
Structural abnormality		411/3776 (10.9%)	411/1177 (34.9%)
Isochromosome	mos 45, X/46, X, i(Xq)/i(Xp)/46, XX	192/3776 (5.0%)	192/1177 (16.3%)
Translocations	t(X;1), t(X;2), t(X;7), t(X;4), t(X;4;10), t(X;7), t(X;8), t(X;12), t(X;14), t(X;15), t(X;17), t(X;19), t(X;20)	42/3776 (1.1%)	42/1177(3.6%)
X-autosomes		23/3776 (0.6%)	23/1177 (1.9%)
Autosomes	t(7;10), t(1;20), t(1;17), t(11;20), t(22;22), t(11;19), t(13;21), t(3;15), t(3;18), t(1;8), t(2;20)	19/3776 (0.5%)	19/1177 (1.6%)
Deletions	46, X, del (Xp)	85/3776 (2.3%)	85/1177 (7.2%)
Xp	46, X, del (Xq)	23/3776 (0.6%)	23/1177 (1.9%)
Xq		62/3776 (1.6%)	62/1177 (5.3%)
Ring chromosome	mos 45, X/46, X, r(X)/46, XX	21/3776 (0.6%)	21/1177 (1.8%)
Marker chromosome	mos 45, X/46, X, +mar/46, XX	22/3776 (0.6%)	22/1177 (1.9%)
Isodicentric	mos 45, X/46, X, idic(X)/46, XX	22/3776 (0.6%)	22/1177 (1.9%)
Other structural abnormalities	Duplication, inversion, etc.	27/3776 (0.7%)	27/1177 (2.3%)

Table 2 Comparison of cytogenetic analysis of present study with the previous findings

References	Total cases	% Abnormal chromosome	Normal (%)	Numerical anomalies (%)	Structural anomalies (%)	Male karyotype (%)
Rajangam and Nanjappa [5]	620	26.1	73.87	11.5	5.6	9.0
Cortes-Gutierrez et al. [15]	187	41.7	58.3	26.7	4.3	10.7
Kalavathi et al. [6]	852	25.8	74.2	11.7	7.04	7.04
Vijayalakshmi et al. [7]	140	27.8	71.2	14.3	7.1	6.4
Yu et al. [13]	340	47.0	53.00	45.2	1.8	–
Faeza [12]	223	20.6	79.37	14.7	4.9	0.9
Samarakoon et al. [3]	338	34.0	66.0	26.0	0.9	6.5
Mohajertehran et al. [11]	180	24.5	75.5	16.7	3.4	4.4
Geckinli et al. [2]	94	37.00	63.00	12.7	11.7	12.7
Stoyanova et al. [10]	140	32.6	61.4	20.7	7.1	6.4
Present study	3776	31.2	68.8	9.7	10.9	10.6

with Stoyanova et al. [10] (32.6%) from Bulgaria, Geckinli et al. [2] (37.0%) from Turkey, Samarakoon et al. [3] (34.0%) from Sri Lanka, whereas Mojahetehran et al. [11] reported 24.5% from Iran and Faeza [12] reported 20.63% from Egypt. Our reported frequency is lower than that in the studies of Yu et al. [13] and Butnariu et al. [14] who reported 47 and 54.6% from China and Romania. From South India, Rajangam and Nanjappa [5], Kalavathi et al. [6] and Vijayalakshmi et al. [7] reported 26.1%, 25.8% and 27.8%, respectively.

Among the different types of chromosomal abnormalities, complete monosomy X ($n = 271/3776$) was observed in 7.2% subjects. The monosomy X is the typical karyotype of Turner syndrome, which is the leading cause of primary amenorrhoea. In the present study, we observed 10.6% cases ($n = 399/3776$) with 46, XY karyotype, which is more than monosomy X (7.2%) ($n = 271/3776$). This trend was observed by Kalavathi et al. [6] and Geckinli et al. [2] also, but the difference is not much as we observed. Studies have demonstrated that a female phenotype can occur in XY embryo when testis determining factor (TDF) or other genes in the testes determining pathway are lost, mutated or compromised [7]. Most of these subjects presented with tall stature, absence of uterus or ovaries, gonadal dysgenesis and androgen insensitivity syndrome. A detailed phenotype–genotype correlation was not possible as some referrals were without precise clinical history. Our centre being a reference laboratory, where we get cases from the peripheral regions of the country and also for the second opinion from the local laboratories for confirmation of the result, we must have received more number of 46, XY female phenotype compared to 45, X karyotype.

In the present study, mosaicism of 45, X/47, XXX was found in 0.9% (33/3776) cases which is lower than that in other studies done by Vijayalakshmi et al. [7] and Geckinli et al. [2] revealing 1.4% and 2.2%. The presence of three X chromosomes could lead to meiotic disturbance which in turn causes ovarian failure [16].

In our study, X-autosome and autosome–autosome translocations were observed in 0.6 and 0.5% (23/3776 and 19/3776) cases respectively. Of this, 18 of 23 X-autosome translocations and 15 of 19 autosome–autosome translocations were already published by us [17, 18]. Balanced autosomal translocations can have deleterious effects on gametogenesis in men and women [19]. The balanced autosomal translocations observed in the present study might cause impairment of oogenesis, leading to primary amenorrhoea.

In our study, Xq deletion and Xp deletion were observed in 1.6 and 0.6% subjects respectively. It was known that deletion of the long arm of the X chromosome in the q13–q27 band leads to ovarian failure and the deficiency of critical growth development gene SHOX on the short arm of X chromosome is the frequent cause of short stature of Turner phenotype [18].

Another interesting abnormality in subjects with clinical features of Turners syndrome in our study was mosaicism of monosomy X with isochromosome X in 5.0% ($n = 192/3776$) cases, which was higher compared to the findings of Kalavathi et al. [6] (2.5%), Mojahetran et al. [11] (0.56%), Faeza [12] (2.7%) and Samarakoon et al. [3] (4.2%), whereas Geckinli et al. [2] found 5.3% in his study group.

In the present study, mosaicism of monosomy X with ring chromosome, marker chromosome and isodicentric chromosome was observed in 1.8, 1.9 and 1.9% cases respectively. This incidence is high when compared to other studies [2, 6].

Apart from the above, we also found derivative X chromosome, inversion of long arm of X chromosome, duplication of Xq, chimerism as 46, XX/46, XY abnormalities in our study as primary amenorrhoea could be due to the involvement of chromosome X. There are other abnormalities like inv 1, 4, 6 and 11, whether these abnormalities have play a role in causing PA is not known or a just de novo abnormality.

Early age referral by careful assessment of the clinical features for the application of cytogenetic investigation and molecular cytogenetics would aid in the delineation of the genetic aetiology in cases of amenorrhoea presenting with abnormal and normal karyotype.

In conclusion, our study is the largest as per the literature available till date and revealed high incidence of chromosomal abnormalities in patients with PA. Hence, patients with amenorrhoea should be initially screened by primary physicians and gynaecologists for non-genetic causes. After exclusion of non-genetic causes, patients with primary amenorrhoea should receive prompt referral for genetic study. If cytogenetic abnormalities are detected, genetic counselling should be given to the patient and the family by a geneticist.

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Compliance with Ethical Standards

Conflict of interest All authors declare that they have no conflict of interest.

Ethical Approval This study has been approved by the SRL Ethical Committee.

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