ORIGINAL ARTICLE
https://doi.org/10.5653/cerm.2023.05848
pISSN 2233-8233 · eISSN 2233-8241
Clin Exp Reprod Med 2023;50(3):192-199

CERM

Clinical profile and cytogenetic correlations in females with primary amenorrhea

Divya Chandel, Priyanka Sanghavi, Ramtej Verma

Department of Zoology, BioMedical Technology and Human Genetics, Gujarat University, Ahmedabad, India

Objective: This study was conducted to investigate chromosomal abnormalities and their correlations with clinical and radiological findings in females with primary amenorrhea (PA).
Methods: Detailed forms were recorded for 470 females, including the construction of three-generation pedigrees. Peripheral venous blood was drawn, with informed consent, for cytogenetic analysis.
Results: An abnormal karyotype was found in 16.38% of participants. The incidence of structural abnormalities (6.8%) exceeded that of numerical abnormalities (6.15%). Turner syndrome represented 45% of all numerical abnormalities. Furthermore, the Y chromosome was detected in 5% of females with PA. Among the structural chromosomal abnormalities detected (n=32) were mosaicism (25%), deletions (12.5%), isochromosomes (18.75%), fragile sites (3.12%), derivatives (3.12%), marker chromosomes (3.12%), and normal variants (29.125%). An examination of secondary sexual characteristics revealed that 29.6% of females had a complete absence of breast development, 29.78% lacked pubic hair, and 36.88% exhibited no axillary hair development. Radiological findings revealed that 51.22% of females had a hypoplastic uterus and 26.66% had a completely absent uterus. Abnormal ovarian development, such as the complete absence of both ovaries, absence of one ovary, one absent and other streak, or both streak ovaries, was observed in 69.47% of females with PA. Additionally 43.1%, 36.1%, 67.4%, and 8% of females had elevated levels of serum follicle-stimulating hormone, luteinizing hormone, thyroid-stimulating hormone, and prolactin, respectively.
Conclusion: This study underscores the importance of karyotyping as a fundamental diagnostic tool for assessing PA. The cytogenetic correlation with these profiles will aid in genetic counseling and further management of the condition.

Keywords: Amenorrhea; Chromosome aberrations; Cytogenetic analysis; Hormones; Mullerian ducts

Introduction

Primary amenorrhea (PA) is defined as the absence of menarche in a female by the age of 14, regardless of whether secondary sexual characteristics (SSCs) are present [1]. The World Health Organization has estimated that 15% of the global population is infertile, with amenorrhea ranking as the sixth leading cause of female infertility [2]. The menstrual cycle is governed by intricate feedback interactions between the hypothalamus, pituitary gland, and ovaries. The primary causes of PA include pituitary-hypothalamic disorders (27.8%), gonadal/ovarian disorders (50.4%), and outflow tract (uterine-vaginal) abnormalities (21.8%) [1]. However, the potential conditions underlying PA are vast and could range from endocrine disorders and genetic abnormalities to psychological, environmental, and structural anomalies [1]. Other contributing factors may be chromosomal disorders or single gene disorders. The incidence of chromosomal abnormalities in PA varies, ranging from 15.9% to 63.3% [2]. Typically, gonadal dysgenesis is caused by X chromosome monosomy (45,X), as seen in Turner syndrome (TS), but amenorrhea can

Received: December 19, 2022 · Revised: April 24, 2023 · Accepted: May 04, 2023
Corresponding author: **Divya Chandel**
Department of Zoology, BioMedical Technology and Human Genetics, Gujarat University, Navrangpura, Ahmedabad 380009, India
Tel: +91-9427633253 Fax: +91-7926857254
E-mail: divya_chandel@yahoo.com

*This study was supported in part by grants from the University Grants Commission under the Department of Special Assistance (DSA) program, Council of Scientific and Industrial Research (CSIR), New Delhi, India and Gujarat State Biotechnology Mission (GSBTM), Government of Gujarat, India.

This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/4.0/) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

© 2023 THE KOREAN SOCIETY FOR REPRODUCTIVE MEDICINE

also occur in 47,XXX trisomy, in pure 46,XX gonadal dysgenesis and 46,XY gonadal dysgenesis (Swyer syndrome), or in intersex disorders (complete androgen insensitivity syndrome or mixed gonadal dysgenesis) [3]. PA is a clinically heterogeneous disease, and if left untreated, it can result in long-term health risks for the patient, including infertility, sexual dysfunction, and a sense of defeminization [4]. Consequently, a systematic, organized approach is necessary for diagnosis, which involves recording physical examination details, conducting a sonogram of the pelvis, performing endocrinological testing, and carrying out karyotyping [5].

The aim of the present study was to determine the frequency of chromosomal aberrations and to identify correlations of the karyotype with the ultrasound (USG) and clinical profiles of females with PA, followed by genetic counseling.

Methods

This study was conducted at the Department of Zoology, Gujarat University, Ahmedabad, where 470 females presenting with PA were referred for cytogenetic analysis. The study considered females aged between 14 and 40 years. Approval was obtained from the University Institutional Ethical Committee (Ref. No. Zool./DHEC-01_2016). A detailed form was completed for each participant, with informed consent provided (for minors, consent was obtained from a parent). The form encompassed a detailed pedigree of at least three generations, information on other family members, the patient's clinical profile (including serum levels of follicle-stimulating hormone [FSH], luteinizing hormone [LH], thyroid-stimulating hormone [TSH], and prolactin), and a comprehensive USG profile. SSCs were evaluated based on the Tanner staging previously described [3]. A detailed USG report was obtained from the clinician at the time of recruitment, and the development of Müllerian duct structures was noted.

For the cytogenetic analysis, peripheral blood lymphocyte culture was performed according to a standard protocol with minor modifications [6]. Venous blood was collected in aseptic conditions, with 0.5 mL added to Roswell Park Memorial Institute (RPMI) 1640 culture medium supplemented with fetal bovine serum. Phytohemagglutinin was introduced into each culture for stimulation, and the cultures were maintained at 37 °C for 72 hours. Colchicine (1 mg/5 mL) was added to arrest the cells at metaphase, followed by treatment with a pre-warmed hypotonic solution (KCl: 0.75M). The cells were fixed in Carnoy's solution (acetomethanol 3:1), and slides were prepared. These slides underwent G-banding [7], with a total of 100 metaphase plates counted to check for mosaicism and 20 plates karyotyped in accordance with International System for Human Cytogenetic Nomenclature 2016. Images were captured by a Zeiss microscope and karyotyping was performed using the Ikaros Imaging System (Metasystems).

Serum hormone levels (FSH, LH, TSH, and prolactin) were assayed via chemiluminescence immunoassay on a Centaur device. The mean values were calculated and categorized as normal, high, or low levels. The reference ranges for hormones were taken from the laboratory values presented in the Endocrine Self-Assessment Program (ESAP, 2015).

Results

1. Cytogenetic findings

Of the 470 females screened for their cytogenetic makeup, 83.61% (n = 393) exhibited a 46,XX karyotype, while 16.38% (n = 77) displayed abnormal karyotypes. The chromosomal abnormalities observed were both structural and numerical (Table 1). The results were divided into X chromosome numerical abnormalities including monosomy X (4.25%), sex reversal (3.4%), and mosaicism (1.9%) (of which 1.7% were mosaic monosomy X and 0.2% were mosaic Y); X chromosome structural abnormalities such as structural mosaicism (1.7%), isochromosome X (1.3%), and deletions in both the p and q arms of the X chromosome (0.85%); normal variants (1.9%) including inv(Y)(qh) (0.85%), inv (9)(qh) (0.42%), 9qh+ (0.42%), and 14ps+(0.21%); and miscellaneous findings (1.1%) including derivatives (0.63%), fragile sites (0.21%), and marker chromosomes (0.21%). Our results indicated a higher percentage of structural abnormalities (6.8%) compared to numerical abnormalities (6.15%).

Table 1. Frequency of chromosomal abnormalities

Cytogenetic category	Karyotype	No. (%)
Normal female karyotype	46,XX	393 (83.6)
Male karyotype	46,XY	16 (3.4)
X numerical	Turner syndrome	20 (4.2)
	Mosaicism (numerical)[a]	9 (1.9)
X structural	Mosaicism (structural)[b]	8 (1.7)
	Deletion[c]	4 (0.8)
	Isochromosome	6 (1.3)
Normal variants	inv(Y)(qh), inv(9)(qh), 9qh+, 14ps+	9 (1.9)
Miscellaneous[d]	Derivative, fragile sites and marker	5 (1.1)
Total		470 (100)

[a]Numerical mosaicism includes: mos 45,X[60]/47,XXX[11]/46,XX[29] (n=4), mos 45,X[53]/46,XX[47] (n=2), mos 45,X[81]/46,XY[19] (n=1), mos 45,X[64]/47,XXY[36] (n=1), mos 46,XY[90]/46,XX[10] (n=1); [b]Structural mosaicism includes: mos 45,X[91]/46,X,+mar[9] (n=2), mos 45,X[71]/46,X,+mar[5]/47,XX,+mar[4]/46,XX[20] (n=1), mos 45,X[85]/46,X,i(X)(q10)[15] (n=3), mos 45,X[62]/46,X,der(X)t(X;X)(p22;p22.3)[38] (n=1), mos 45,X[78]/46,X,i(X)(q10)[12]/46,X,del(X)(q10)[8]/47,X,i(X)(q10),+mar[2] (n=1); [c]Deletion in the X chromosome includes: 46,X,del(X)(q13) (n=2), 46,X,del(X)(p22.2) (n=1), 46,X,del(X)(q26) (n=1); [d]Miscellaneous karyotype includes: 46,X,der(X)del(X)(q13q22)inv(X)(q23q27) (n=1), 46,XX,der(10)t(10;15)(p15;q22.1) (n=1), 46,XX,inv(9)(qh),del(18)(q12q21) (n=1), 46,XX,fra(16)(q22) (n=1), 47,XX,+mar (n=1).

2. Secondary sexual characters and ultrasonography of Müllerian duct

The development of SSCs correlated with the females' karyotypes (Table 2). Breast development was examined in 155 females with PA. Of these, 36.78% (n=57) reported normal breast development, 33.5% (n=52) had underdeveloped breasts, and 29.6% (n=46) showed complete absence of breast development. The development of pubic and axillary hair was evaluated in 141 females with PA and categorized as present, absent, or sparse/scanty. Well-developed axillary hair was seen in 39% (n=55), absent in 36.8% (n=52), and sparse/scanty in 24.1% (n=34). Pubic hair was well-developed in 41.1% (n=58), absent in 29.7% (n=42), and sparse/scanty in 29% (n=41).

Ultrasonography screening was performed on 285 females. Of these, the uterus was present in 22.1% (n=63), absent in 26.66% (n=76), and found to be hypoplastic in 51.22% (n=146) of females with PA (Table 3). Among the 146 females with a hypoplastic uterus, 120 exhibited a normal 46,XX female karyotype, confirming a diagnosis of Mayer–Rokitansky–Küster– Hauser (MRKH) syndrome. Examination of the ovaries showed that both ovaries were present in 30.52% (n=87) of females, both were absent in 29.8% (n=86), one was absent in 14.38% (n=41), one was absent and the other was streak in 6.31% (n=18), and both were streak ovaries in 18.6% (n=53). The high number of females with a hypoplastic uterus and normal ovaries indicated that MRKH syndrome was a significant cause of PA. A total of 16 females were screened for the development of male genitalia (testes), of which 11 showed the presence of testes (Table 4). The females who exhibited the presence of testes were

Table 2. Development of secondary sexual characteristics in different cytogenetic categories

Cytogenetic category	Breast development (n=155)			Pubic hair (n=141)			Axillary hair (n=141)		
	Developed	Underdeveloped	Not developed	Present	Sparse/scanty	Absent	Present	Sparse/scanty	Absent
Normal female	49 (31.6)	40 (25.8)	34 (21.9)	49 (34.7)	33 (23.4)	31 (21.9)	48 (34.0)	27 (19.1)	39 (27.6)
Male karyotype	2 (1.3)	4 (2.6)	2 (1.3)	2 (1.4)	2 (1.4)	3 (2.1)	2 (1.4)	1 (0.7)	3 (2.1)
X numerical[a]	2 (1.2)	3 (1.9)	6 (3.8)	2 (1.4)	1 (0.7)	7 (4.9)	2 (1.4)	1 (0.7)	7 (4.9)
X structural[b]	3 (1.9)	2 (1.2)	3 (1.9)	4 (2.8)	3 (2.1)	-	2 (1.4)	3 (2.1)	2 (1.4)
Normal variants[c]	1 (0.6)	2 (1.2)	1 (0.6)	1 (0.7)	1 (0.7)	1 (0.7)	1 (0.7)	1 (0.7)	1 (0.7)
Miscellaneous[d]	-	1 (0.6)	-	-	1 (0.7)	-	-	1 (0.7)	-
Total	57 (36.7)	52 (33.5)	46 (29.6)	58 (41.1)	41 (29.0)	42 (29.7)	55 (39)	34 (24.1)	52 (36.8)

Values are presented as number (%).

[a]Numerical mosaicism includes: mos 45,X[60]/47,XXX[11]/46,XX[29] (n=4), mos 45,X[53]/46,XX[47] (n=2), mos 45,X[81]/46,XY[19] (n=1), mos 45,X[64]/47,XXY[36] (n=1), mos 46,XY[90]/46,XX[10] (n=1); [b]Structural mosaicism includes: mos 45,X[91]/46,X,+mar[9] (n=2), mos 45,X[71]/46,X,+mar[5]/47,XX,+mar[4]/46,XX[20] (n=1), mos 45,X[85]/46,X,i(X)(q10)[15] (n=3), mos 45,X[62]/46,X,der(X)t(X;X)(p22;p22.3)[38] (n=1), mos 45,X[78]/46,X,i(X)(q10)[12]/46,X,del(X)(q10)[8]/47,X,i(X)(q10),+mar[2] (n=1); [c]Deletion in the X chromosome includes: 46,X,del(X)(q13) (n=2), 46,X,del(X)(p22.2) (n=1), 46,X,del(X)(q26) (n=1); [d]Miscellaneous karyotype includes: 46,X,der(X)del(X)(q13q22)inv(X)(q23q27) (n=1), 46,XX,der(10)t(10;15)(p15;q22.1) (n=1), 46,XX,inv(9)(qh),del(18)(q12q21) (n=1), 46,XX,fra(16)(q22) (n=1), 47,XX,+mar (n=1).

Table 3. Radiological findings of the uterus and ovarian development in different cytogenetic categories

Cytogenetic findings	Uterus development (n=285)			Ovarian development (n=285)				
	Present (n=63)	Hypoplastic (n=146)	Absent (n=76)	Present (n=87)	One absent and another streak (n=18)	Only one absent (n=41)	Streak/small (n=53)	Absent (n=86)
Normal female (n=231)	56 (19.6)	120 (42.1)	55 (19.2)	80 (28.0)	16 (5.6)	36 (12.6)	40 (14.0)	59 (20.7)
Normal male (n=15)	1 (0.3)	5 (1.7)	9 (3.1)	1 (0.3)	-	4 (1.4)	4 (1.4)	6 (2.1)
X numerical (n=19)[a]	4 (1.4)	9 (3.1)	6 (2.1)	5 (1.7)	-	-	5 (1.7)	9 (3.1)
X structural (n=13)[b]	1 (0.3)	10 (3.5)	2 (0.7)	1 (0.3)	2 (0.7)	-	4 (1.4)	6 (2.1)
Normal variants (n=6)[c]	1 (0.3)	1 (0.3)	4 (1.4)	-	-	1 (0.3)	-	5 (1.7)
Miscellaneous (n=1)[d]	-	1 (0.3)	-	-	-	-	-	1 (0.3)
Total (n=285)	63 (22.1)	146 (51.2)	76 (26.6)	87 (30.5)	18 (6.3)	41 (14.3)	53 (18.6)	86 (29.8)

Values are presented as number (%).

[a]Numerical mosaicism includes: mos 45,X[60]/47,XXX[11]/46,XX[29] (n=4), mos 45,X[53]/46,XX[47] (n=2), mos 45,X[81]/46,XY[19] (n=1), mos 45,X[64]/47,XXY[36] (n=1), mos 46,XY[90]/46,XX[10] (n=1); [b]Structural mosaicism includes: mos 45,X[91]/46,X,+mar[9] (n=2), mos 45,X[71]/46,X,+mar[5]/47,XX,+mar[4]/46,XX[20] (n=1), mos 45,X[85]/46,X,i(X)(q10)[15] (n=3), mos 45,X[62]/46,X,der(X)t(X;X)(p22;p22.3)[38] (n=1), mos 45,X[78]/46,X,i(X)(q10)[12]/46,X,del(X)(q10)[8]/47,X,i(X)(q10),+mar[2] (n=1); [c]Deletion in the X chromosome includes: 46,X,del(X)(q13) (n=2), 46,X,del(X)(p22.2) (n=1), 46,X,del(X)(q26) (n=1); [d]Miscellaneous karyotype includes: 46,X,der(X)del(X)(q13q22)inv(X)(q23q27) (n=1), 46,XX,der(10)t(10;15)(p15;q22.1) (n=1), 46,XX,inv(9)(qh),del(18)(q12q21) (n=1), 46,XX,fra(16)(q22) (n=1), 47,XX,+mar (n=1).

https://doi.org/10.5653/cerm.2023.05848

Table 4. Radiological findings of testes and penis development in different cytogenetic categories

Cytogenetic findings	Testes (n = 16)		Penis (n = 16)		
	Present	Absent	Present/Small	Absent	Hypospadias
46,XX (n = 9)	6	3	4	-	5
45,X (n = 1)	-	1	-	-	1
46,XY (n = 3)	2	1	2	1	-
mos 46,XY/46,XX (n = 1)	1	-	1	-	-
46,X,inv(Y)(qh) (n = 2)	2	-	2	-	-
Total (n = 16)	11	5	9	1	6

found to have chromosomal abnormalities: 46,XY (n = 2), mosaic 46,XY/46,XX (n = 1), and 46,X,inv(Y) (qh) (n = 2). The presence of testes in females with a Y chromosome complement confirmed a diagnosis of androgen insensitivity syndrome.

3. Endocrinology findings

The mean serum levels of FSH (n = 174), LH (n = 152), TSH (n = 92), and prolactin (n = 100) were analyzed and compared with cytogenetic findings (Table 5). The mean values for each hormone were categorized as normal, high, or low. The serum FSH levels of 174 females with PA were analyzed, revealing that 43.1% (n = 75) had high FSH levels, 40.8% (n = 71) were within the normal range, and 16.1% (n = 28) had low FSH levels. Serum LH levels were examined in 152 females. Of these, 51.3% (n = 78) had normal LH levels, 36.1% (n = 55) had high LH levels, and 12.5% (n = 19) exhibited low LH levels. TSH levels were assessed in 92 females. The results showed that 15.2% (n = 14) fell within the normal range, 67.4% (n = 62) had high levels, and 17.4% (n = 16) had low TSH levels. Serum prolactin levels were measured in 100 females, revealing that 85% (n = 85) had normal levels, 8% (n = 8) had high levels, and 7% (n = 7) had low prolactin levels.

Discussion

Several etiological factors such as anatomical defects, hormonal imbalances, genetic elements, and environmental influences are known to cause PA. The most helpful classification for identifying the causes of PA is based on the presence or absence of SSCs and internal female genitalia. Genetic factors, which could include single gene disorders and chromosomal abnormalities, significantly contribute to the etiology of PA. The assessment of chromosomal abnormalities is often considered by clinicians for an accurate diagnosis of PA. Therefore, evaluating these abnormalities and providing genetic counseling for females is crucial for managing amenorrhea. The frequency of chromosomal abnormalities reported in previous studies ranges from 14% to 42% (Table 6) [1,2,4,5,8-18].

In our study, we categorized chromosomal abnormalities in PA as either X numerical, X structural, the presence of Y chromosome complement, or normal variants. The structural abnormalities of the X chromosome observed included deletions, inversions, isochromosomes, and derivatives of the X chromosome. We also observed other abnormalities such as mosaicism, marker chromosomes, and normal variants. Interestingly, the frequency of structural abnormalities was higher than that of numerical abnormalities, a finding that contrasts with the study by Korgaonkar et al. [8]. This discrepancy might be attributable to differences in selection size, presentation timing, or geographical region.

1. Cytogenetic evaluation

In our study, the majority of females displayed a normal female karyotype, confirming diagnoses of either pure gonadal dysgenesis or MRKH syndrome [19]. We observed that these females developed SSCs and had normal gonadotropin levels. The frequency of normal karyotypes in our study falls within the previously reported range (65% to 85%) for the Indian population [1,2,8-16].

Monosomy of the X chromosome is a typical karyotype of TS, where affected females commonly display clinical features such as a webbed neck, short stature, cubitus valgus, and infantilism [20]. This condition arises due to non-disjunction events that occur during meiosis or mitosis, which are believed to cause either pure or mosaic TS [21]. In our study, we observed complete monosomy of the X chromosome in 4.25% (n = 20) of females and X chromosome mosaicism in 3.4% (n = 16) of females. These results align well with previous studies that identified TS as a major cause of PA [1,8,13,16,17,21]. These findings further underscore the importance of the genes on the X chromosome for normal female physiology and reproduction [16].

An isochromosome involving the long arm of the X chromosome, a variant of TS, was detected in 1.3% (n = 6) of females. The presence of this isochromosome aligns with the explanation that isochromosomes appear to be metacentric with identical arms, leading to partial deletion of Xp genes and partial trisomy of Xq genes. This imbalance can cause partial or complete ovarian failure [1]. Another variant of TS that we observed involves a deletion in the long arm of the

Clin Exp Reprod Med 2023;50(3):192-199

Table 5. Clinical profiles in patients with different cytogenetic findings

Cytogenetic findings	FSH (μIU/mL) (n=174)			LH (μIU/mL) (n=152)			TSH (μIU/mL) (n=92)			Prolactin (ng/mL) (n=100)		
	Low (n=28)	Normal (n=71)	High (n=75)	Low (n=19)	Normal (n=78)	High (n=55)	Low (n=16)	Normal (n=14)	High (n=62)	Low (n=7)	Normal (n=85)	High (n=8)
46,XX	0.8±0.5	7.1±5	112.8±85	0.1±0.1	5.8±4.5	99±194.5	0.05±0.1	2.2±1	35.1±48	2.8±0.9	13.5±6.9	299.8±508.8
46,XY	0.2[e]	7.1±4.6	58.9±14.4	0.1[e]	5.3±0.4	50.3±42.2	-	2.8±1.6	9.9[e]	-	13.8±7.5	-
X numerical[a]	-	8.9[e]	108.4±47.6	-	11.5±5.9	57.9±56.2	0.14[e]	2.5±1.5	21.9[e]	-	12.3±2.3	90[e]
X structural[b]	-	-	71±29.8	-	12.6±5.2	55.3±39.2	0.1[e]	2.4±0.2	12.6±10.7	3.2[e]	15.04±8.2	35[e]
Normal variants[c]	-	4.4±2.2	72.4[e]	-	3.8±0.3	-	-	2.3±1.7	-	-	6.4[e]	-
Miscellaneous[d]	-	24[e]	-	-	18[e]	-	-	-		-	10[e]	-
Total	28 (16.1)	71 (40.8)	75 (43.1%)	19 (12.5)	78 (51.3)	55 (36.1)	16 (17.4)	14 (15.2)	62 (67.4)	7 (7)	85 (85)	8 (8)

Values are presented as mean±standard deviation or number (%).
FSH, follicle-stimulating hormone; LH, luteinizing hormone; TSH, thyroid-stimulating hormone.
[a]Numerical mosaicism includes: mos 45,X[60]/47,XXX[11]/46,XX[29] (n=4), mos 45,X[53]/46,XX[47] (n=2), mos 45,X[81]/46,XY[19] (n=1), mos 45,X[64]/47,XXY[36] (n=1), mos 46,XY[90]/46,XX[10] (n=1); [b]Structural mosaicism includes: mos 45,X[91]/46,X,+mar[9] (n=2), mos 45,X[71]/46,X,+mar[5]/47,XX,+mar[4]/46,XX[20] (n=1), mos 45,X[85]/46,X,i(X)(q10)[15] (n=3), mos 45,X[62]/46,X,der(X)t(X;X)(p22;p22.3)[38] (n=1), mos 45,X[78]/46,X,i(X)(q10)[12]/46,X,del(X)(q10)[8]/47,X,i(X)(q10),+mar[2] (n=1); [c]Deletion in the X chromosome includes: 46,X,del(X)(q13) (n=2), 46,X,del(X)(p22.2) (n=1), 46,X,del(X)(q26) (n=1); [d]Miscellaneous karyotype includes: 46,X,der(X)del(X)(q13q22)inv(X)(q23q27) (n=1), 46,XX,der(10)t(10;15)(p15;q22.1) (n=1), 46,XX,inv(9)(qh),del(18)(q12q21) (n=1), 46,XX,fra(16)(q22) (n=1), 47,XX,+mar (n=1); [e]n=1 mean values cannot be calculated.

chromosome, specifically in the Xq13-26 region, which results in premature ovarian failure (POF). Studies have suggested that the genes *POF1* (localized to Xq21.2-q27 or within Xq26.1-q27) and *POF2* (localized to Xq13-q21.1) are responsible for ovarian dysgenesis [2,17]. In this study, 1.06% of females (n=5, of whom one female showed structural mosaicism, three females showed structural deletion, and one female displayed a derivative of the X chromosome) had a deletion at Xq10-26, a finding consistent with the regions associated with the suggested POF genes.

A significant percentage of patients with PA showed the presence of a Y chromosome, which could be attributed to sex reversal or androgen insensitivity. We observed that females with testicular feminization did not exhibit development of the Müllerian duct and might possess functioning male gonads. Therefore, the presence of testes increases the risk of malignancy and necessitates surgical removal [3]. Further support for this comes from the study by Ghosh et al. [12], which suggested that the sex determining region Y (*SRY*) gene on the Y chromosome initiates the differentiation of bi-potential gonads into testes. However, in the absence or reduced expression of the SRY gene, these bi-potential gonads differentiate into inappropriate female gonads. This finding underscores the importance of early diagnosis for the proper management of the condition.

Translocation (10;15)(p15;q22.1) was another unique chromosome alteration observed in one of the females in the present study. This suggests that a deletion in the aldo-keto reductase family 1 member C1 (*AKR1C1*) gene, located on 10p15-14, may be responsible for POF, although the exact cause remains uncertain. Another gene, cytoplasmic polyadenylation element-binding protein 1 (*CPEB1*), situated on 15q25.1, regulates the proteins involved in forming the synaptonemal complex during oocyte maturation. It also regulates mitotic cell progression in the S and M phases, which is crucial for embryonic cell division. Haploinsufficiency of the *CPEB1* gene can lead to early germ cell loss during a woman's reproductive years, a condition that was reported in only one female who presented with POF and showed a microdeletion in 15q25.1 [22]. The translocation between 10p15 and 15q22.1 provides clear evidence that the *AKR1C1* and *CPEB1* genes play a significant role in the development of PA. To our knowledge, the translocation (10;15)(p15;q22.1) has not been documented in any previous studies on PA.

Fragile sites are regions of chromatin that fail to compact during mitosis. The fragile site FRA16B, presented in our study, has been associated with reproductive disorders, including recurrent pregnancy loss, spontaneous premature delivery, menarche and menopause complications, ovulatory dysfunction, endometriosis, and in some cases, ovarian, breast, and uterine cancers [23]. To date, only two studies have reported the presence of fragile sites in females with PA and secondary amenorrhea, suggesting that fra(16)(q22) may be re-

https://doi.org/10.5653/cerm.2023.05848

Table 6. Comparison of previous cytogenetic studies with the present study

Study	Population studied	Year	No. of PA cases	No. of normal karyotype (%)	No. of abnormal karyotype (%)
Ganguly et al. [11]	India	2003	280	200 (71)	80 (29)
Wong et al. [17]	Hongkong	2005	237	179 (75.5)	58 (24.5)
Rajangam et al. [15]	India	2007	620	458 (73.8)	162 (26.1)
Cortes-Gutierrez et al. [5]	Mexico	2007	187	109 (58.2)	78 (41.7)
Vijayalakshmi et al. [16]	India	2010	140	101 (72.2)	39 (27.8)
Ayatollahi et al. [18]	Iran	2010	220	176 (80)	44 (20)
Kalavathi et al. [13]	India	2010	852	632 (74.1)	220 (25.8)
Charania et al. [10]	India	2010	74	61 (82.4)	13 (17.5)
Merin et al. [14]	India	2012	246	210 (85.3)	36 (14.6)
Tanmahasamut et al. [4]	Thailand	2012	295	236 (80)	59 (20)
Dutta et al. [2]	India	2013	637	505 (79.2)	132 (20.7)
Amin et al. [9]	India	2014	98	78 (79.5)	20 (20.5)
Malla et al. [1]	India	2016	108	70 (64.8)	38 (35.1)
Ghosh et al. [12]	India	2018	150	114 (76.1)	36 (23.9)
Korgaonkar et al. [8]	India	2018	490	369 (75.3)	121 (24.7)
Present study	India	2019	470	393 (83.6)	77 (16.3)

PA, primary amenorrhea.

sponsible for PA [15,23].

We also observed a significant percentage of normal variants (1.9%) in female participants, challenging the hypothesis that normal variants have no significant effect on the phenotype of PA [15]. Earlier studies have reported the presence of normal variants at similar percentages, offering an opportunity to further investigate the role of these variants in the occurrence of PA [8].

2. Secondary sexual characteristics and ultrasonography evaluation

Utilizing imaging studies to assess the Müllerian duct provides multiplanar, sensitive, and specific images, which have proven to be reliable predictors for evaluating Müllerian abnormalities [19,24]. The absence of a uterus and ovaries is typically regarded as one of the clinical features of PA [3]. Our study found that 26.6% (n = 76) of participants lacked a uterus and 51.22% (n = 146) had a hypoplastic uterus, indicating significant anatomical abnormalities. These findings aligned with those reported in earlier studies [8,21]. Another notable observation from our study was the presence of testes in females with a Y chromosome complement. This strongly suggests that testicular feminization had occurred due to the secretion of Müllerian inhibitory factors, leading to the regression of internal Müllerian structures and resulting in cryptorchid testicular tissue without androgenization [25]. Therefore, the surgical removal of the testes is recommended immediately upon detection to prevent future malignancies.

Examining the development of SSCs is another crucial aspect of assessing PA. Breast development is a marker for normal estrogen production [26]. We attempted to correlate the development of SSCs with PA and found that 36.7% (n = 57) of the females displayed normal breast development. This suggests that PA in these females might be due to androgen resistance or the congenital absence of the uterus [3], indicating MRKH syndrome. These findings were more frequent than those reported in previous studies [8,21]. The absence of SSCs is also an important marker for investigating PA. Lobo [3] classified the etiology of PA with absent SSCs as being due to gonadal failure, hypothalamic failure, or pituitary failure. Our study found that 63.2% (n = 98) of females exhibited underdeveloped or absent SSCs, thus indicating ovarian failure before the onset of puberty or gonadal dysgenesis [27]. These findings align with the classification provided for the initial examination of PA [3].

3. Endocrinological evaluation

Assessing serum gonadotropins alongside USG imaging forms the primary diagnostic process for evaluating PA. In the absence of SSCs, it is advisable to examine the serum levels of FSH, LH, TSH, and prolactin. Elevated levels of FSH and LH suggest hypergonadotropic hypogonadism, and karyotype analysis typically reveals either 45,X cell lines (indicative of TS) with or without mosaicism, pure 46,XY cell lines (indicative of Swyer syndrome), or 46,XX (indicative of POF) [27]. Studies were conducted to understand the role of gonadotropins in PA and found that all females with PA showed an increase in mean levels of FSH and LH, likely due to an insufficient sample size and estimation of only a single gonadotropin [1,8]. In our study, we observed that 43.1% (n = 75) of females had elevated serum FSH and 36.1% (n = 55) had elevated serum LH. This suggests that increased gonadotropin activity causes hypergonadotropic hypogonadism, an important risk factor for developing PA. Studies indicate that hyper-

gonadotropic hypogonadism primarily occurs due to mutations in genes coding for gonadotropin receptors located in female ovaries [25,27-31]. We also observed normal or low levels of gonadotropins, indicating hypogonadotropic hypogonadism, which suggests hypothalamic or pituitary dysfunction. This finding challenges the two-gonadotropin two-cell hypothesis, which posits that LH stimulates ovarian thecal cells to produce androgens, which act as precursors for estrogens in granulosa cells, where FSH induces aromatization to estrogens [28].

The study by Fazeli and Nachtigall [32] suggested that high serum TSH (hypothyroidism) is a risk factor for PA in females as it can lead to pituitary gland enlargement, thus causing hyperprolactinemia, including amenorrhea. It is known that up to 30% of amenorrheic females may have pre-existing or will develop hypothyroidism, which could also be a possible cause of POF due to polyglandular autoimmune syndromes. However, it's challenging to detect due to the poor sensitivity of current assays [33]. We sought to investigate the association of hypothyroidism with PA and found that 67.4% (n = 62) of females presented with hypothyroidism. This contrasts with the findings of Kallepalli and Kallepalli [34], where none of the 58 females showed hypothyroidism. This evidence suggests that TSH plays a significant role in developing PA, leading to the hypothesis that high levels of TSH could increase susceptibility to PA.

Research has shown that high serum prolactin can disrupt the normal secretion of gonadotrophin-releasing hormone and subsequently decrease estrogen levels, thereby leading to amenorrhea. In our study, we explored the potential association of serum prolactin (prolactin) levels with PA. It has been suggested that up to 60% of cases with high prolactin levels, often due to pituitary tumors, can be detected by magnetic resonance imaging, with dopamine agonists preferred for treatment [35]. However, in our study, only 8% (n = 8) of the females exhibited high prolactin levels. Out of these, six females had a 46,XX karyotype, which clearly signifies hyperprolactinemia. This supports the aforementioned observation that high levels of serum prolactin are the most common pituitary cause of amenorrhea [36].

In conclusion, cytogenetic evaluation serves as the cornerstone for identifying the etiology, with the inclusion of other genetic aspects. The discovery of novel karyotypes could open doors to understanding the mechanisms involved. Correlating cytogenetic findings with clinical aspects can aid clinicians in pinpointing the exact cause of this condition, whether it be POF, hypothyroidism, or hyperprolactinemia. Genetic counseling, coupled with various management tools, can provide a comprehensive understanding and psychological support to alleviate the burden on the individual and her family.

Conflict of interest

No potential conflict of interest relevant to this article was reported.

ORCID

Divya Chandel https://orcid.org/0000-0002-2047-9253

Author contributions

Conceptualization: DC. Data curation: DC. Formal analysis: DC. Funding acquisition: DC, RV. Methodology: DC. Project administration: DC, RV. Visualization: DC. Writing-original draft: DC, PS. Writing-review & editing: DC, PS, RV.

References

1. Malla TM, Dar FA, Pandith AA, Zargar MH. Frequency and pattern of cytogenetic alterations in primary amenorrhea cases of Kashmir, North India. Egypt J Med Hum Genet 2016;17:25-31.
2. Dutta UR, Ponnala R, Pidugu VK, Dalal AB. Chromosomal abnormalities in amenorrhea: a retrospective study and review of 637 patients in South India. Arch Iran Med 2013;16:267-70.
3. Lobo RA. Primary and secondary amenorrhea and precocious puberty: etiology, diagnostic evaluation, management. In: Karz VL, Lentz GM, Lobo RA, Gershenson DM. editors. Comprehensive gynecology. 5th ed. Mosby; 2007. p. 933-61.
4. Tanmahasamut P, Rattanachaiyanont M, Dangrat C, Indhavivadhana S, Angsuwattana S, Techatraisak K. Causes of primary amenorrhea: a report of 295 cases in Thailand. J Obstet Gynaecol Res 2012;38:297-301.
5. Cortes-Gutierrez EI, Davila-Rodriguez MI, Vargas-Villarreal J, Cerda-Flores RM. Prevalence of chromosomal aberrations in Mexican women with primary amenorrhoea. Reprod Biomed Online 2007;15:463-7.
6. Hungerford DA. Leukocytes cultured from small inocula of whole blood and the preparation of metaphase chromosomes by treatment with hypotonic KCl. Stain Technol 1965;40:333-8.
7. Seabright M. A rapid banding technique for human chromosomes. Lancet 1971;2:971-2.
8. Korgaonkar S, Dhangar S, Kulkarni V, Kerketta L, Vundinti BR. Clinical and cytogenetic profile of 490 cases of primary amenorrhea. J Med Sci Clin Res 2018;6:487-94.
9. Amin SV, Rai L, Palpandi P, Kumaran A. Ever intriguing 'primary amenorrhea': an audit. Int J Reprod Contracept Obstet Gynecol

https://doi.org/10.5653/cerm.2023.05848

2014;3:1090-6.
10. Charania J, Khan A. Cytogenetic study in patients with menstruation disorders. Internet J Hum Anat 2010;2:1-5.
11. Ganguly BB, Sahni S. X chromosomal abnormalities in Indian adolescent girls. Teratog Carcinog Mutagen 2003;Suppl 1:245-53.
12. Ghosh S, Roy S, Pal P, Dutta A, Halder A. Cytogenetic analysis of patients with primary amenorrhea in Eastern India. J Obstet Gynaecol 2018;38:270-5.
13. Kalavathi V, Chandra N, Nambiar GR, Shanker J, Sugunashankari P, Meena J, et al. Chromosomal abnormalities in 979 cases of amenorrhea: a review. Int J Hum Genet 2010;10:65-9.
14. Merin T, Rema D, Preetha T, Amudha S, Jayalakshamma J, Mary M. Amenorrhea: cytogenetic studies and beyond. Am J Mol Cell Biol 2012;1:25-32.
15. Rajangam S, Nanjappa L. Cytogenetic studies in amenorrhea. Saudi Med J 2007;28:187-92.
16. Vijayalakshmi J, Koshy T, Kaur H, Mary FA, Selvi R, Parvathi VD, et al. Cytogenetic analysis of patients with primary amenorrhea. Int J Hum Genet 2010;10:71-6.
17. Wong MS, Lam ST. Cytogenetic analysis of patients with primary and secondary amenorrhoea in Hong Kong: retrospective study. Hong Kong Med J 2005;11:267-72.
18. Ayatollahi H, Safaei A, Vasei M. Cytogenetic analysis of patients with primary amenorrhea in Southwest of Iran. Iran J Pathol 2010; 5:121-6.
19. Shweta Thapa KC, Cunjian Y. Mayer Rokitansky Kuster Hauser syndrome: a review article. Int J Sci Invent Today 2017;6:553-61.
20. Turner HH. A syndrome of infantilism, congenital webbed neck, and cubitus valgus. Endocrinology 1938;23:566-74.
21. Kara N, Tural S, Elbistan M, Karakus N, Guven D, Kocak I. Cytogenetic findings of patients with Amenorrhea in Turkish population: a retrospective study. Int J Hum Genet 2012;12:87-92.
22. McGuire MM, Bowden W, Engel NJ, Ahn HW, Kovanci E, Rajkovic A. Genomic analysis using high-resolution single-nucleotide polymorphism arrays reveals novel microdeletions associated with premature ovarian failure. Fertil Steril 2011;95:1595-600.
23. Bhavani G, Sivaprakash S, Samuel CR, Santhiya ST. Enhanced expression of FRA16B using AT-rich DNA binding chemicals in a woman with secondary amenorrhoea. J Clin Diagn Res 2017;11: QD01-3.
24. Sultan C, Biason-Lauber A, Philibert P. Mayer-Rokitansky-Kuster-Hauser syndrome: recent clinical and genetic findings. Gynecol Endocrinol 2009;25:8-11.
25. Basak S, Prakash A. Investigation and treatment of primary amenorrhoea. Obstet Gynaecol Reprod Med 2013;23:364-9.
26. Klein DA, Poth MA. Amenorrhea: an approach to diagnosis and management. Am Fam Physician 2013;87:781-8.
27. Child T. Investigation and treatment of primary amenorrhoea. Obstet Gynaecol Reprod Med 2011;21:31-5.
28. Bhagavath B, Layman LC. Genetics of female infertility in humans. In: Emery AEH, Korf BR, Rimoin DL, Pyeritz RE. editors. Emery and Rimoin's principles and practice of medical genetics. 6th ed. Elsevier Inc.; 2014. p. 1-24.
29. Cordts EB, Christofolini DM, Dos Santos AA, Bianco B, Barbosa CP. Genetic aspects of premature ovarian failure: a literature review. Arch Gynecol Obstet 2011;283:635-43.
30. Master-Hunter T, Heiman DL. Amenorrhea: evaluation and treatment. Am Fam Physician 2006;73:1374-82.
31. McCabe MJ, Dattani MT. Genetic aspects of hypothalamic and pituitary gland development. Handb Clin Neurol 2014;124:3-15.
32. Fazeli P, Nachtigall LB. Hyperprolactinemia and pituitary causes of amenorrhea. In: Santoro N, Neal-Perry G. editors. Amenorrhea. Springer; 2010. p. 83-100.
33. Hayden CJ, Balen AH. Primary amenorrhoea: investigation and treatment. Obstet Gynaecol Reprod Med 2007;17:199-204.
34. Kallepalli P, Kallepalli D. A study of primary amenorrhea cases in north coastal Andhra Pradesh. Ment Retard 2019;2:3-44.
35. Golden NH, Carlson JL. The pathophysiology of amenorrhea in the adolescent. Ann N Y Acad Sci 2008;1135:163-78.
36. Practice Committee of the American Society for Reproductive Medicine. Current evaluation of amenorrhea. Fertil Steril 2006; 86(5 Suppl 1):S148-55.