DOI: 10.7860/JCDR/2022/59500.17116

Original Article

Obstetrics and Gynaecology Section

Proportion and Pattern of Chromosomal Abnormalities in Primary Amenorrhea in Kerala- A Retrospective Study

UMA THANKAM[1], SANKAR VAIKOM HARIHARAN[2], SANTHI SAROJAM[3], REMYA SYAMALA[4], SHERRIN THOMAS ALEX[5], NANDINI V RATNAMMA[6], SUJAMOL JACOB[7]

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ABSTRACT

Introduction: Primary amenorrhoea may be due to chromosomal abnormalities and identification of these abnormalities is important for counselling and management of these individuals.

Aim: To identify the prevalence of chromosomal abnormalities in a cohort of primary amenorrhoea patients and to evaluate the various pattern of chromosomal abnormalities.

Materials and Methods: This retrospective study was conducted at Government Medical College, Thiruvananthapuram, Kerala, India (serves as a referral centre for most of south Kerala and adjoining districts of Tamil Nadu), from January 2013 to December 2020. All phenotypically females, in the age group of 15-30 years, attending the Genetic Clinic with a diagnosis of primary amenorrhoea were evaluated with karyotype from peripheral blood as per the standard protocol. An abstraction proforma was used to collect the data from the master case sheet available in the genetic laboratory. Cytogenetic abnormalities were described as per the standard International System for Human Cytogenomic Nomenclature (ISCN). For statistical analysis, proportion of cases with chromosome abnormality in the cohort was described as percentage.

Results: Chromosomal analysis revealed 25.5% (38 out of 149) with abnormal karyotype. Among the abnormalities, the most common abnormality was 45, X (12, 31.6%) Turners syndrome. Other abnormalities included sex reversal female (46, XY) in six (15.8%), isochromosome Turner syndrome in five (13.1%), partial deletion in X chromosome in three (7.8%) and various combination of mosaic pattern in nine (23.7%) cases. Hypergonadotropic hypogonadism was significantly associated with chromosomal abnormality.

Conclusion: Cytogenetic abnormality is a cause for primary amenorrhoea in a significant proportion of cases and karyotype should be an integral part of evaluation in such cases. In resource limited settings, karyotype is having more clinical utility in cases with hypergonadotropic hypogonadism.

Keywords: Isochromosome, Karyotype, Mosaicism, Sex reversal, Turner syndrome

INTRODUCTION

Primary amenorrhoea is a condition when a girl fails to attain menarche. When a girl has no secondary sexual characteristics by the 13 years of age or she does not attain menarche even after five years of initial breast development nor at 15 years or more, she has to be evaluated for primary amenorrhoea [1]. Major causes of primary amenorrhoea include genetic and chromosomal abnormalities leading on to ovarian dysfunction, hypothalamic or pituitary problems, congenital abnormalities of the reproductive tract and physical problems leading to reproductive dysfunction. Apart from physical examination, imaging and hormonal profile, genetic testing especially karyotype should be done for definitive diagnosis [2]. Identification of the cause of amenorrhoea is important for the specific management and counselling of the patients and family.

The frequency of chromosomal abnormalities varies from 14-42% in primary amenorrhoea in various studies [3,4]. A recent study from North Kerala showed chromosomal abnormalities in 32.08% of patients with primary amenorrhoea [5]. While the previous study has identified the proportion of chromosomal abnormalities in primary amenorrhoea in North Kerala, this study from South Kerala with wider and varied population area made an attempt to bring about a clinico-endocrine relation to the chromosome abnormalities. The aim of this study was to determine the proportion of patients with chromosomal abnormalities in patients with primary amenorrhoea attending the Genetic Clinic at a tertiary care hospital situated in South Kerala, India.

MATERIALS AND METHODS

This retrospective study was conducted at a tertiary care hospital, Government Medical College, Thiruvananthapuram, Kerala, India (serves as a referral centre for most of Southern Kerala and adjoining districts of Tamil Nadu), from January 2013 to December 2020. The Institutional Ethical Committee clearance was obtained for the study (HEC No 09/04/2021/MCT dated 12.11.2021).

Inclusion criteria: All phenotypic females, in the age group 15-30 years, attending the Genetic Clinic/Laboratory for karyotype, with a diagnosis of primary amenorrhoea were included in the study. Karyotyping in all the primary amenorrhoea patients was the algorithm. Total number of patients referred for cytogenetic studies during this period were 150.

Exclusion criteria: Individuals where cytogenetic study result was not available due to culture failure were excluded from the study (one person had a culture failure).

Finally, 149 patients were included in the analysis of the chromosomal abnormalities. Details of patients were obtained using Data Abstraction forms while deidentifying the personal information. An abstraction proforma was used to collect the data from the master case sheet available in the Genetic Laboratory. In cases where no chromosomal abnormalities were identified, other possible causes of primary amenorrhoea were abstracted from the data sheet wherever available. Patients with elevated Follicle Stimulating Hormone (FSH) and Luteinising Hormone (LH) was considered as hypergonadotropic hypogonadism. Other hormones like Thyroid Stimulating Hormone (TSH), Triiodothyronine (T3), Thyroxine (T4), prolactin and oestradiol values were also collected. All hormones were tested in chemiluminescent method. The imaging of the uterus and ovaries (ultrasound and magnetic resonance imaging) were also taken wherever results were available.

For cytogenetic analysis, 2 mL peripheral blood sample was collected in heparinised vacutainers from all the patients. Conventional cytogenetic analysis was performed by phytohemagglutinin stimulated lymphocyte micro culture method [6]. Lymphocytes were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium for 72 hrs. Metaphase were arrested by adding colchicine 2 hrs prior to harvesting. Harvesting was performed using the hypotonic potassium chloride (KCl) solution followed by fixation using 3:1 methanol-acetic acid mix. Samples were washed and slides for metaphase study were prepared, banded and stained using trypsin and Giemsa stain. For each patient, 20 G-banded metaphases at 400-550 band resolution were studied and in case of mosaicism 30 metaphases were karyotyped at ×1000 magnification using a Olympus' new BX53 microscope and analysis was performed using Applied Spectral Imaging (ASI) software. Results were recorded using the International System for Human Cytogenetic Nomenclature (ISCN) 2016 [7].

STATISTICAL ANALYSIS

The proportion of patients with chromosomal abnormality was calculated as percentage. Various chromosomal abnormalities were described as proportions. The association of chromosomal abnormality with hypergonadotropic hypogonadism was calculated using Chi-square test. A p-value <0.05 was considered as statistically significant.

RESULTS

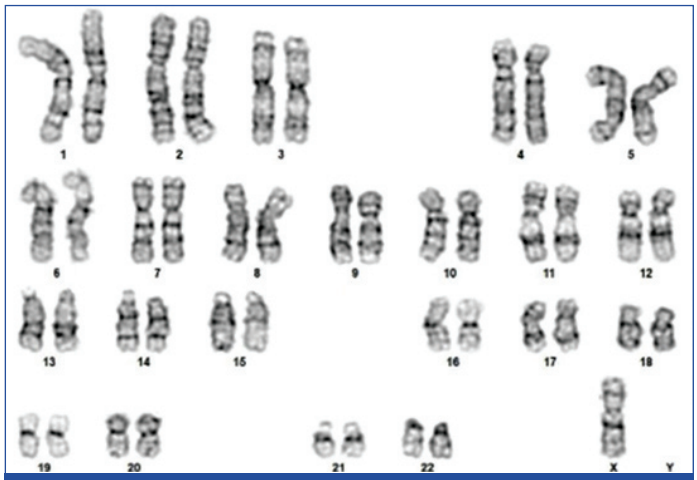
A total of 150 primary amenorrhoea cases were referred for conventional cytogenetic analysis in 8 years with a median age of 17 years. Of this, chromosomal result was available for 149 cases (one culture failure) and were included in the analysis. The other clinical features identified in primary amenorrhoea subjects were short stature, phenotypic features of Turner syndrome (cubitus valgus, webbing of neck), and absence or poorly developed secondary sexual characters. Few subjects had dysmorphic features, mental retardation and structural malformations of other organs.

Chromosomal analysis revealed 25.5% (38 of 149 cases) with abnormal chromosomal complement, 71.8 % (107 of 149 cases) with normal chromosomal constitution and 2.7% (4 of 149) with polymorphic chromosomal variations which were considered as normal [Table/Fig-1].

Type of abnormality	Karyotype		Total number of cases 149 (%)
Normal	46, XX		107 (71.8)
Abnormal			38 (25.5)
Normal Variations			4 (2.7)
		n (%) out of total number of cases (n=149)	n (%) out of abnormal cases (n=38)
Numerical abnormality			
Turner syndrome (TS)	45, X	12 (8.0)	12 (31.6)
Triple X syndrome	47, XXX	1 (0.67)	1 (2.7)
Turner mosaic	46, XX/ 45, X	2 ((1.3)	2 (5.2)
	46, XY/45, X	2 (1.3)	2 (5.2)
Structural abnormality			
TS/ Deletion	46, X, del (X) (p21p21)	3 (2.0)	3 (7.8)
	46, X, del (X) (q21pter)		
	46, X, del (X) (q25)		
Isochromosome	46, X, iso (X) (q10)	5 (3.3)	5 (13.2)
Mosaic	45, X/46, Iso (X) (q10)	4 (2.7)	4 (10.5)
Isodicentric chromosome	45, X/46, X, idic (X)	1 (0.67)	1 (2.6)
Sex-autosome translocation	45, XX, t (13;21)	1 (0.67)	1 (2.6)
Marker chromosome	47, XX, mar+	1 (0.67)	1 (2.6)
XY female	46, XY	6 (4.0)	6 (15.8)
Normal variations	46, XX, inv (9)	1 (0.67)	1 (2.6)
	46, XX, 21ps+	2 (1.3)	2 (5.3)
	46,XX, 9qh+	1 (0.67)	1 (2.6)

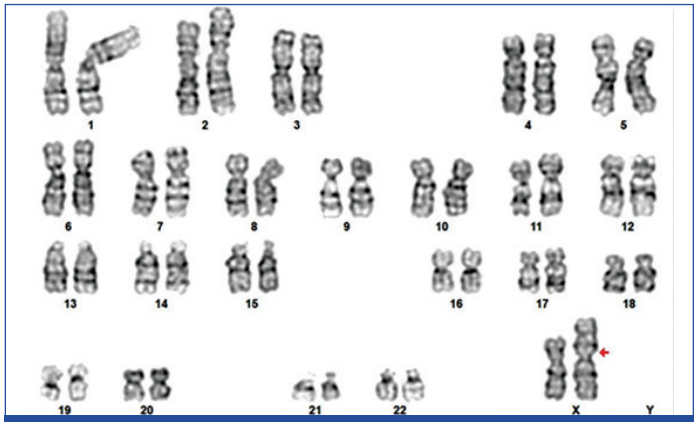
[Table/Fig-1]: Chromosomal analysis of patients with primary amenorrhoea (N=149).

[Table/Fig-2] showed that 45, X (31.6%, 12 of 38) was the most frequent abnormality in the study. The second most common abnormality seen among these patients with primary amenorrhoea was sex reversal female (46, XY) in 15.8% (6 of 38).



[Table/Fig-2]: Karyotype of a patient with Turner syndrome. Karyotype showing deletion of one sex chromosome [45, X].

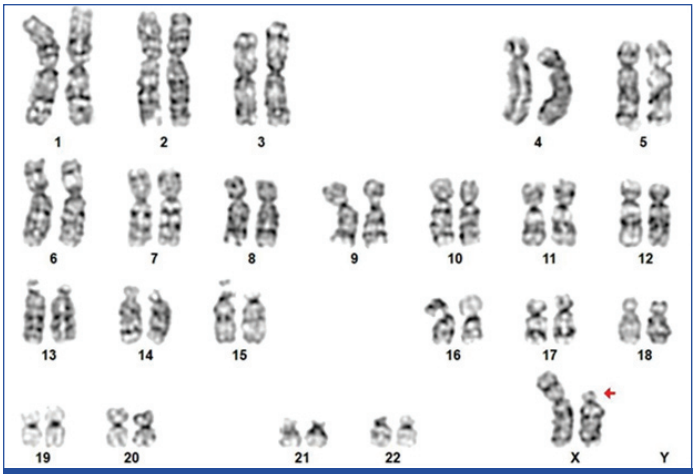
Other chromosomal abnormalities of Turner syndrome identified were isochromosome in five cases (13.1%) [Table/Fig-3], partial deletion of X chromosome in three cases (7.8%) [Table/Fig-4] and various mosaic pattern in nine cases (23.7%). Other abnormalities like 47, XXX, structural abnormality of autosomes and marker chromosome were also seen in one case each.



[Table/Fig-3]: Karyotype of Isochromosome. Karyotype showing isochromosome (Isoq) (deletion of short arm with duplication of long arm of X chromosome).

Hormonal profile identified 59 subjects with hypergonadotropic hypogonadism, of these 37 had chromosomal abnormality (62.7%). Whereas, among 90 patients with hypogonadotropic hypogonadism only one patient had chromosomal abnormality. Total 97% of person with chromosomal abnormality had hypergonadotropic hypogonadism (p-value <0.0001). There was a significant association between hypergonadotropic hypogonadism and chromosomal abnormality.

Other major causes of primary amenorrhoea apart from chromosomal abnormalities were constitutional delay in growth and puberty in 19.4% (29/149), Müllerian agenesis in 13.4% (20/149), ovarian dysgenesis/failure with normal karyotype in 11.4% (17/149), Polycystic Ovarian Disease (PCOD) in 6% (9/149), thyroid disorders in 5.3% (8/149) and syndromic causes in 4.6% (7/149).



[Table/Fig-4]: Structural abnormality of chromosome. Karyotype showing partial deletion of short arm of X chromosome {46, X, del(X)}.

DISCUSSION

Primary amenorrhoea is seen in 2-5% of women of child bearing age. Genetic disorders like gonadal dysgenesis and Mullerian agenesis are attributed to half of the cases in most of the studies. Karyotype is the important investigation in female with primary amenorrhoea to identify any chromosomal abnormality [8]. In the present study, proportion of chromosomal abnormality in women in the age group 15-30 years were studied; 25.5 % cases had chromosomal abnormalities, which was comparable to other studies [Table/Fig-5] [3-5,9-18].

Study	Year	Country	N	Abnormal Karyotype
Rajangam S and Nanjappa L, [9]	1973-2005	India	620	26.13 %
Cortés-Gutiérrez EI et al., [4]	1995-2003	Mexico	187	41.71 %
Wong MS [10]	1991-2002	Hongkong	237	24.50%
El-Dahtory F, [11]	2008-2010	Egypt	223	20.63%
Kalavathy V et al., [12]	1979-2004	India	852	25.80%
Vijayalakshmi J et al., [13]	2010	India	140	27.80%
Samarakoon L et al., [14]	2005-2011	Sri Lanka	338	34%
Ghosh S et al., [15]	2013-2015	India	150	23.90%
Koppaka NT et al., [16]	2001-2016	India	3776	31.20%
Elkarhat Z et al., [17]	1996-2016	Morocco	161	17.39%
Pal AK et al., [3]	2000-2017	India	174	13.22%
Soltani N et al., [18]	2011-2019	Iran	200	29%
Lekha S et al., [5]	2014-2020	India	53	32.08%
Present study	2013-2020	India	149	25.05%

[Table/Fig-5]: Comparison of cytogenetic analysis of present study with literature [3-5,9-18].

Prevalence of chromosomal abnormalities in females with primary amenorrhoea from Sri Lanka showed 34% [14] and Mexico showed 41.7% [4] which is higher than that of Indian population whereas it was seen to be lower in studies from Morocco (17.4%) [17] and Hong Kong (24.5%) [10].

The major abnormality identified in this study was aneuploidy of X chromosome (45, X and 47, XXX) and structural abnormality involving the X chromosome like partial deletion and isochromosome. Mosaic pattern of various combinations were also identified in eight cases. The present study identified sex reversal female karyotype (46, XY) presenting as primary amenorrhoea in six cases. One case of translocation involving 13 and 21 chromosomes also had primary amenorrhoea. The distribution frequencies of different abnormal karyotypes have similar frequencies in other studies [3-18]. Sex reversal karyotype (46, XY) is seen less commonly in Indian population compared to other studies, which necessitates a confirmation in a larger sample size.

In the current study, one patient with primary amenorrhoea had 47, XXX karyotype without any mental retardation but had ovarian failure. A similar case has been reported previously in non mosaic cases of 47, XXX [19].

The 46, XY in a phenotypically female with primary amenorrhoea may be due to Swyer syndrome or androgen insensitivity syndrome. Swyer syndrome is a female phenotype with normally developed Müllerian ducts, streak gonads, poorly developed breast and primary amenorrhoea [20]. Male sexual differentiation is initiated by the *SRY* gene (sex determining region of Y chromosome). Sex reversed female may be due to deletion or mutation in the *SRY* gene present in the Y chromosome. Whereas, in androgen insensitivity syndrome, gonads (testis) will be present in the inguinal region or abdomen and the individual will be having a blind ending vagina. This is due to mutation in the androgen receptor (X-linked) failing to develop as male external genitalia. In the present study, two patients were confirmed with androgen insensitivity syndrome and rest may had Swyer syndrome.

Among the 149 patients, 59 patients had hypergonadotropic hypogonadism suggestive of ovarian dysgenesis. Chromosomal abnormality was identified in 37 cases of ovarian dysgenesis. Among 38 cases with chromosomal abnormality, 37 had hypergonadotropic hormonal profile which had a significant association statistically. Based on this it was recommend that, in a resource poor setting where karyotype is not easily available, clinician can decide on the utility of karyotype based on hormonal profile. Also, all females presenting with primary amenorrhoea and a hypergonadotropic hormonal profile should have a karyotype done at the earliest.

Among individuals with hypogonadotropic hypogonadism, 29 patients had constitutional delay in puberty and they had their menarche later. Among others, four had thyroid disorders, nine had polycystic ovarian diseases, 12 had Müllerian abnormalities and five had developmental/syndromic causes.

Autosomal genes have a crucial role in reproductive development and mutations in Gonadotrophin Releasing Hormone (GnRH) receptor, beta subunit of Follicle Stimulating Hormone (FSHβ), Follicle Stimulating Hormone Receptor (FSHR), and Luteinising Hormone Receptor (LHR) gene mutations can lead to primary amenorrhoea. Consanguinity has been reported to be the primary etiological factor resulting in homozygous condition for recessive deleterious genes [21]. It was observed that four patients with parental consanguinity in this cohort and all these individuals had ovarian dysgenesis without any cause. Further, testing like exome sequencing is required in these patients to identify the causative gene [22].

Management of primary amenorrhoea depends on the aetiological diagnosis and appropriate intervention [23]. A hormonal therapy for such patients to promote timely development of secondary sexual characters is important in the long-term management. Primary amenorrhoea due to chromosomal abnormalities and Mullerian agenesis may lead to anxiety, depression and suicidal tendencies in affected individuals. Genetic counselling and psychological support to the individual and family should be an integral part of the management in these patients. During counselling, mechanism of origin of the anomaly, the recurrence risk, the hormonal therapy, education/hobby/career, marriage and reproductive options to be considered.

Limitation(s)

As it was a referral clinic-based study, it may not reflect the actual scenario in the community. Also, the study requires special technical expertise and hence, cannot be done at a primary or secondary level healthcare centre. A karyotype alone cannot identify all genetic causes for primary amenorrhoea. Advanced genetic testing like whole exome sequencing in women with ovarian failure and a normal karyotype may provide a larger insight into the genetic causes.

CONCLUSION(S)

In this study there was 25.5% chromosomal abnormalities in patients with primary amenorrhoea. Identifying this abnormality is essential for a definitive diagnosis, appropriate counselling and their subsequent management. However, in a resource poor setting, as a normal karyotype was seen in 98% of hypogonadotropic hypogonadism and abnormality was seen in 61.2% of hypergonadotropic hypogonadism, a karyotype may be restricted to hypergonadotropic conditions. Other investigations like thyroid dysfunction and imaging (MRI) of pelvis should also be considered for the aetiological diagnosis.

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PARTICULARS OF CONTRIBUTORS:

1. Associate Professor, Department of Obstetrics and Gynaecology, Government Medical College, Thiruvananthapuram , Kerala, India.
2. Additional Professor, Department of Paediatrics, Government Medical College, Thiruvananthapuram, Kerala, India.
3. Cytogeneticist, Department of Genetic Laboratory, Child Development Centre, Thiruvananthapuram, Kerala, India.
4. Cytogeneticist, Department of Genetic Laboratory, Child Development Centre, Thiruvananthapuram, Kerala, India.
5. Genetic Councillor, Department of Genetic Laboratory, Child Development Centre, Thiruvananthapuram, Kerala, India.
6. Professor, Department of Obstetrics and Gynaecology, Government Medical College, Thiruvananthapuram, Kerala, India.
7. Professor and head, Department of Obstetrics and Gynaecology, Government Medical College, Thiruvananthapuram, Kerala, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Sankar Vaikom Hariharan,
House no- TC 50/1641, SIVADAM, Thaliyal, Karamana (PO) Trivandrum,
Thiruvananthapuram, Kerala, India.
E-mail: sankarvh@gmail.com

PLAGIARISM CHECKING METHODS: [Jain H et al.]

- Plagiarism X-checker: Aug 05, 2022
- Manual Googling: Oct 13, 2022
- iThenticate Software: Oct 18, 2022 (14%)

ETYMOLOGY: Author Origin

AUTHOR DECLARATION:

- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? NA
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **Aug 04, 2022**
Date of Peer Review: **Sep 07, 2022**
Date of Acceptance: **Oct 20, 2022**
Date of Publishing: **Nov 01, 2022**