Original Article

Received on 30.07.2016, **Accepted on** 10.08.2016

Indian Journal of Anatomy
Volume 5 Number 3, September - December 2016
DOI: http://dx.doi.org/10.21088/ija.2320.0022.5316.21

Clinical and Cytogenetic Study in Primary Amenorrhea

Vinayak Kulkarni*, SeemaKorgaonkar, Babu Rao Vundinti*****

Abstract

The failure to menstruate by the age of 16 years in the presence of normal secondary sexual characters or 14 years in the absence of other evidence of puberty defines primary amenorrhea and warrant investigations. Though its incidence is less than 0.1%, there is great challenge for clinician in compartmentalizing its etiology and cytogenetics, along with hormonal profile plays a major role in classification of primary amenorrhea. We have carried out a study in 100 cases of primary amenorrhea to evaluate the clinical and cytogenetic correlation in patients with primary amenorrhea. The patient's clinical details were recorded in the case record sheet. The chromosome preparation was done from the peripheral blood according to standard protocol. The chromosome preparations were subjected to GTG banding and karyotyped according to the International System for Human Cytogenetic Nomenclature (ISCN 2011). The fluorescence in situ hybridization (FISH) was carried out using centromeric probes for X and Y chromosome as per the standard protocol. Of the several different causes of PA, anatomical abnormality was seen in 26% cases, ovarian failure in 47% cases and remaining 27% cases were because of constitutional delay and other factors. The chromosomal aberrations were detected in 28% cases. The present study has emphasized that cytogenetic study (karyotyping and FISH) is one of the fundamental investigations for the diagnosis and management of primary amenorrhea.

Keywords: Primary Amenorrhea (PA); Cytogenetics; Fluorescence in Situ Hybridization (FISH) Chromosomal Abnormalities (CA); Karyotype; Secondary Sexual Characters; Follicle Stimulating Hormone (FSH); Ultrasonography (USG).

Introduction

The primary amenorrhea (PA) is defined as absence of menstruation by the age of 16 years in the presence of normal secondary sexual characters or 14 years in the absence of other evidence of puberty. Primary amenorrhea is not a disease but a symptom that may result from several different causes.These are 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 CNS (hypothalamic-pituitary dysfunction) [1]. Incidence of PA is 15% (WHO), the sixth largest major cause of female infertility [2]. Overall it is estimated that endocrine disorders account for approximately 40% of the causes of PA, with the remaining 60% having developmental (genetic or structural) origins [3]. Chromosomal abnormalities are found to be the most important factor in the causation of PA hence the genetic basis of it should be revealed. There is lot of variation in thefrequency of chromosomal abnormalities in primary amenorrhea and the other caseswith normal female karyotype may be associated with abnormal or absent gonads and hypoplastic or absent uterus.

In this study we have analysed the types of chromosomal abnormalities seen in primary amenorrhea and correlated these with different clinical and hormonal factors.

Author's Affiliation: *Associate Professor, Department of Anatomy, LTMMC & GH, Sion, Mumbai, Maharashtra **Technical Officer, ***Scientist E, Department of Cytogenetics, NIIH, Parel, Mumbai, Maharashtra.

Corresponding Author: Vinayak Vilasrao Kulkarni, A/7, Anand Bhavan Quarters, Nair Hospital Campus, Mumbai Central, Mumbai-400011.

E-mail: dr.vinayakkulkarni@gmail.com

Materials and Methods

100 cases of primary amenorrhea coming to our institute from Mumbai and surrounding region for cytogenetic analysis during the period of 2012 to 2015 were enrolled in the study. During presentation their age ranged from 14 to 30 years. After informed written consent from the patient, details like age, height, clinical features breast development, pubic hairs, axillary hairs, hormone profile and USG details were recorded in the case record sheet. Also at the time of referral consent was obtained from each patient or relative, that the information may be presented or discussed or published without revealing the identity of the patient.

Chromosomal preparation was done from the peripheral blood based on a short-term culture of activated T-lymphocytes stimulated with phytohemagglutinin. After sampling of 3-5 ml of peripheral blood in sodium heparin vacutainer tube; blood cells (T-lymphocites) were cultured (37° for 72 hours) on RPMI 1640 medium (9 ml), supplemented with fetal calf serum (1 ml), L - glutamine (0.1 ml), phytohemagglutinin and antibiotics (penicillin and streptomycin). Then, the culture was treated with a hypotonic solution, fixed, and dropped onto a microscope slide.The slides were stained and examined on optical microscope, direct or after application of G- banding (after trypsin treatment) and karyotyped according to International System for Human Cytogenetic Nomenclature (ISCN 2011).

The metaphases were analysed with a Nikon microscope and images were captured with an automated image analysis system. For each case a minimum 30 metaphases were analysed.

A standard protocol was followed for FISH. The slides with metaphase chromosomes prepared as per standard cytogenetic procedure were dehydrated in 70%, 80% and 100% ethanol for 2 min each at room temperature and air dried. The centromeric, locus specific probes for X &Y chromosomes were used for FISH. Probes were mixed with hybridization buffer and deionized distilled water and applied to the slides. The metaphase chromosomes and the probes were co-denatured using Hybrite at 73°C for 3 minutes. The slides were sealed with coverslip using rubber cement and hybridization was carried out for 24 hours at 37°C. After 24 hours of hybridization, the coverslip was removed and the slides were rinsed in formamide wash solution (0.4X SSC / 0.3%NP-40) at 45°C and the slides were air dried. After air drying, the slides were counterstained with DAPI (7.5µl / slide), covered with coverslip, stored in dark prior to signal enumeration and observed under fluorescent microscope for appropriate signals.

Results

Distribution of clinical features such as absence of breast development, absent secondary sexual characters and stature were presented in Figure 1. In the present study 42% cases of PA presented as short stature. Frequency of abnormal breast development has been noticed in57% cases, pubic hairs was absent in 59 % cases and axillary hairs was absent in 61% cases of PA.The FSH levels (>20 mIU / ml) were found to be increased in 63 cases of PA.

The data on different causes such as mullerian agenesis, AIS, vaginal septum, imperforate hymen,

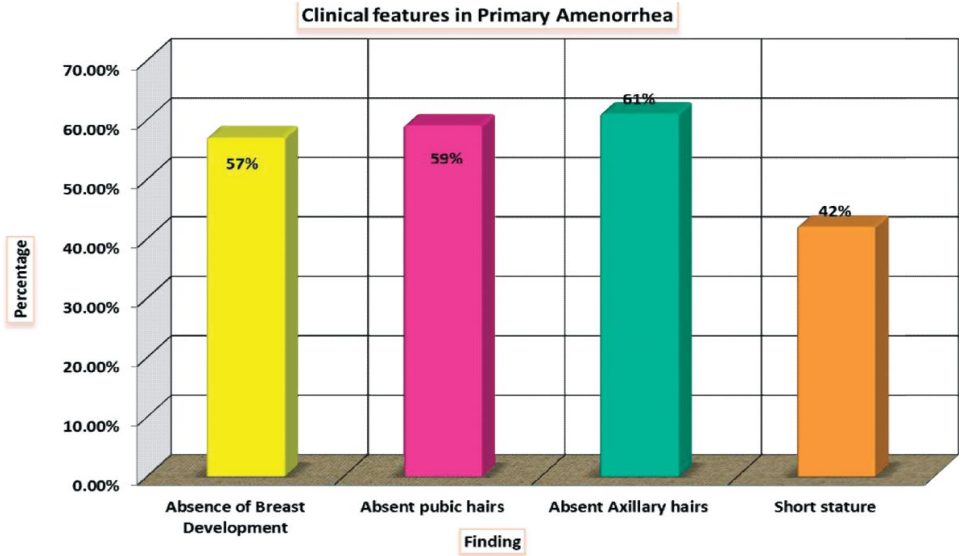

Fig. 1: Distribution of clinical features in PA

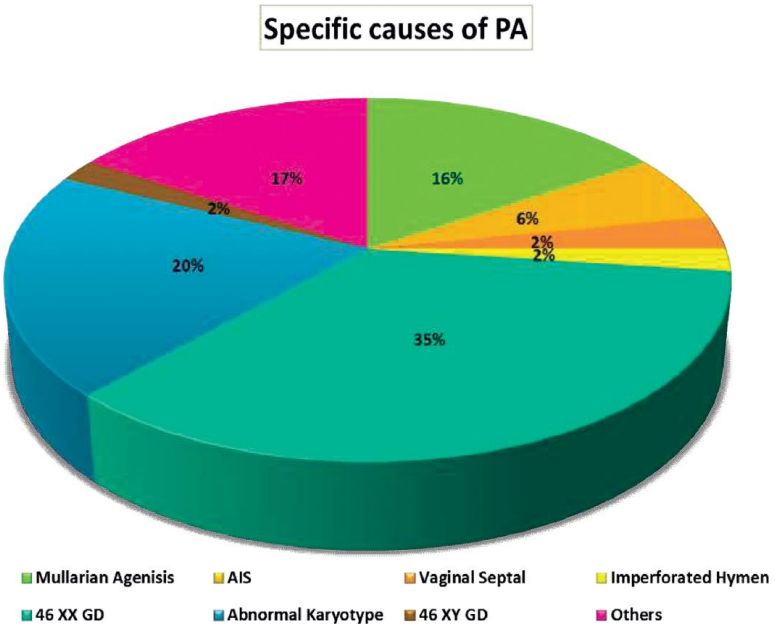

Fig. 2: Distribution of etiological factors in PA

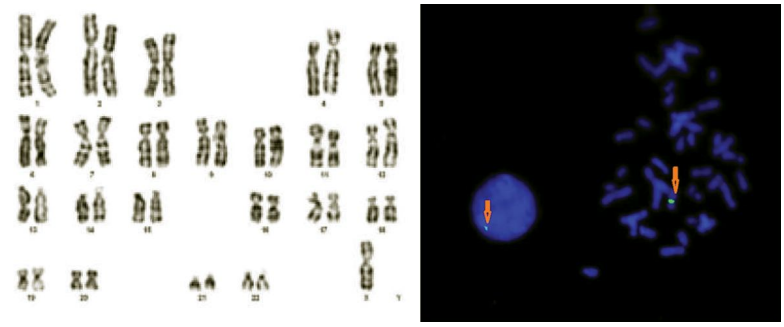

Fig. 3: Karyotype and FISH showing Monosomy X

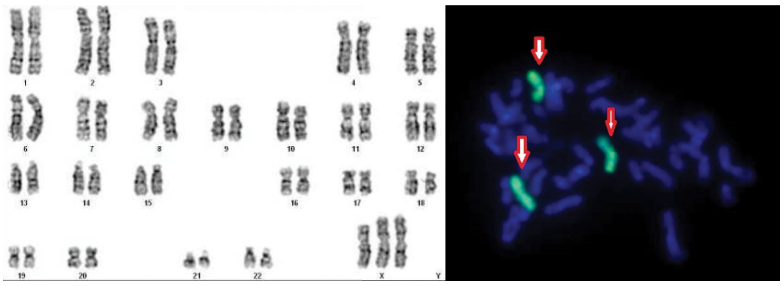

Fig. 4: Karyotype and FISH showing iso(Xq)

Table 1: Distribution of chromosomal abnormality in PA

Karyotype	No. of cases	Percentage
45,X	9	28%
45,X/46,XX	4	
46,X,i(Xq)& 47,X,i(q),i(q)	3	
46,X,del(X)	2	
46,X,der(X)t(X;10)q11:q11)	1	
46,X,X,inv9(p11:q13)	1	
46,XY	8	
46,XX	72	72%

ovarian failure due to 46,XX, 46,XY and abnormal karyotype is presented in figure 2.Among the several different causes ofPA, frequency of ovarian failure due to increased FSH levels is seen to be high in 46,XX GD in 35% cases, 46,XY GD in 8 %cases, abnormal karyotype in 20 % cases andanatomical factor such as mullerian agenesis is found in 16% cases, imperforate hymen in 2% , vaginal septum in 2% cases and other causes such as constitutional delay in 17% cases.

In this study, frequency of the chromosomal aberrations such as 45,X was found in 9% cases, followed by 46,XY in 8%, 45,X/46,XX in 4% 46,X,i(Xq) & 47,X,i(q),i(q) in 3%, 46,X,del(X) in 2%, 46,X,der(X) t(X;10)(q11.q11) in 1%; 46,XX,inv9(p11.q13) in 1% (Table 1).

Discussion

In the present study of Primary amenorrhea, it has been reported that genetic factors accounts of 28 % cases of amenorrhea which indicates that apart from endocrinological and structural causes, cytogenetic abnormality is playing important role.Hence knowledge of cytogeneticsis essential for further management of these cases. The aim of the present study was to find out the role of the cytogeneticsin PA and to correlate the cytogenetic abnormality with clinical and hormonal factors . Assessment of secondary sexual characters like breast development, development of pubic and axillary hairs plays important role as it reflects the normal gonadal activity. The patients having normal secondary sexual characters, pelvic USG is advised to identify presence of uterus, cervix and upper vagina (which rule out mullerian agenesis) and ovaries (which rule out gonadal dysgenesis).The reported incidence of PA in the previous studies ranges from 16-78% and the average frequency of CA in women with PA

was around 42% [4]. Lakshmi Kalpanaet al [5] found abnormal karyotype in 20 / 70(28.57%)cases, Mondel et al [6] in 24 / 72(33.33%) cases, Vijayalaksmi et al [7] in 39 / 140(27.85%) cases andRajangam et al [3] in 162 / 620(26.13%) cases of PAin Indian population. In the present study, out of the 100 cases of PA, normal female karyotype was seen in 72% cases and abnormal karyotype is seen in 28% cases.

Broadly the chromosomal abnormality in the PA cases are grouped as pure Turner (45,X), X mosaicism, structural abnormality and 46,XY female. In the present study the distribution of the CA in PA cases is found to be, 45,X (9%), X mosaicism (4%), structural abnormality of X and other chromosome (7%) and 46,XY female (8%) (Table 1). Those females who present as PA with karyotype 46,XY are phenotypically female since the abnormal gonadal tissue in these cases fails to producemullerian inhibiting factor and testosterone. Gonadal tumours occur in up to 25% of women with a Y chromosome; unlike complete androgen sensitivity syndrome, these gonads do not secrete hormones and should be removed at the time of diagnosis [8]. As the incidence of male karyotype in this study is high, the presence of Y chromosome should be confirmed by molecular cytogenetic technique like FISH. At the same time these cases needs to be further assessed for any mutation of SRY gene, SF1 gene and the other genes which are responsible for male karyotype in phenotypic female with PA [9].

Conclusion

The present study concluded that cytogenetic abnormalities plays an important rolein women with the absence of menstruation and secondary sexual characters. Hence these women should be investigated for chromosomal abnormality along with the routine hormonal and radiological (USG) investigations for the exact diagnosis and management. More emphasis should be given to cytogenetic investigations as the clinical signs and symptoms are found to be variable in these cases. After exclusion of non-genetic causes, patients with PA should receive prompt referral for genetic and molecular study. Genetic counselling should include the risk of premature menopause for patients with Turner's syndrome, possibility of pregnancy in cases with X mosaicism and the use of hormone replacement therapy, the risk of gonadal malignancy for patients with XY GD and the possibility of infertility in patients with other chromosomal aberrations.

References

1. SperoffL, Fritz MA, eds. Amenorrhea. Clinical gynaecologic endocrinology and infertility, 7th edn. Philadelphia: Lippincott Williams. 2005; p.401-464.
2. World health organization, Special Programme of Research Development and Research training in Human Reproduction.1985 14th Annual report, Geneva. 1985; 114: 119.
3. Catherine j Hayden. Primary amenorrhea: Investigation and treatment. Obstetrics, gynaecology and reproductive medicine 17.7, 2007 Elsevier Ltd.: 199-204.
4. Rajangam S, Nanjappa L. Cytogenetic studies in amenorrhea. Saudi Med J. 2007; 28(2): 187-192.
5. LakshimiKalpana V, Satyanarayana M. Cytogenetic Analysis of Primary Amenorrhea Cases. Ind J Hum Genet. 1997; 3(2): 81-91.
6. Mondel SK, Guha D, Banerjee D, Sinha SK. Study of primary amenorrhea with special reference to cytogenetic evaluation. Indian J Pathol Microbiol. 2002 Apr; 45(2): 155-9.
7. Vijayalakshmi J, Koshy T, Kaur H, Andrea F, Selvi R, DeepaParvathi et al. Cytogenetic Analysis of Patients with Primary Amenorrhea. Int J Hum Genet. 2010; 10(1-3): 71-76.
8. Van Niekerk WA. Chromosomes and the Gynaecologist. Am J ObstetGynecol. 1978; 130(8): 291-292.
9. PreetiPaliwal, Anshul Sharma, Shweta Birla, AlkaKriplani et al. Identification of novel SRY mutations and SF1 (NR5A1) changes in patients with pure gonadal dysgenesis and 46,XY karyotype. Molecular Human Reproduction. 2011; 17(6): 372-378.