Cytogenetic studies in amenorrhea

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ABSTRACT

Objective: To study the frequency of the chromosomal abnormality (CA), referred for karyotyping, and counseling in individuals with primary amenorrhea (PA) and secondary amenorrhea (SA).

Methods: We report on a retrospective survey of 865 women with amenorrhea (620-PA and 245-SA) at the Division of Human Genetics, Department of Anatomy, St. John's Medical College, Bangalore, India from 1973 to 2005.

Results: The frequency of the CA in amenorrhea was 23.35%, while PA was 26.13%, and SA was 16.33%. Numerical CA was prevalent in 45.54% of the total; 43.83% in PA, and 52.5% in SA. In numerical chromosomal abnormality, the observed karyotypes were: 45,X; 47,XXX; X mosaicism (45,X/46,XX; 45,XX/46,XX/47,XXX; 45,X/47,XXX; 46,XX/47,XXX); Y mosaicism (45,X/46,XY; 45,X/47,XYY); and others: 46,XX/47,XX+10; 46,XX/46,XY; 46,XX/47,XXY. In addition, is the presence of 46,XY female condition in 63 cases (31.19%), out of which 34.57% were detected to be associated with primary, and 17.5% with SA. Included in the structural chromosomal anomaly were: 46,X,i(Xq); reciprocal translocation [46,XX,t(9;14)]; Robertsonian translocation (13;14); X; autosomal translocations (X;12 and X;14); deletion/duplication/ fragment/isochromosome/marker/ring formation associated either with the long or the short arms of X chromosome; 46,XX,9q-; 46,XX/46,XX,3p(break); in a pure free status or mostly in mosaic status.

Conclusion: The present study has emphasized that karyotyping is one of the fundamental investigations in the evaluation of amenorrhea. It has highlighted CA, one of the genetic etiology as the causal factor in amenorrhea.

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Amenorrhea is defined as no menstruation by age 14 in the absence of growth or development of secondary sexual characteristics, no menstruation by age 16 regardless of the presence of normal growth and development with the appearance of secondary sexual characteristics and in a woman who has been menstruating, the absence of menstruation for a length of time equivalent to a total of at least 3 of the previous cycle intervals, or 6 months of amenorrhea.[1] Traditionally amenorrhea has been classified as primary (PA) or secondary amenorrhea (SA). It may be noted that management depends on the needs of the individual cases. Amenorrhea is one of the major problems encountered by women. This problem may lead to anxiety, depression, and suicidal tendencies in the affected individuals. Since the psychological and social impact is high, patients seeking genetic evaluation are a small fraction of affected women, and hence the exact incidence or prevalence of amenorrhea is not known. The World Health Organization has estimated 15% of the human population as being infertile and amenorrhea as the sixth largest major cause of female infertility. Among the general population, amenorrhea seemed to have affected 2-5% of all women of childbearing age.[2] The etiology of amenorrhea has been compartmentalized as disorders of the outflow tract/ovary/anterior pituitary/CNS factors, and with genetic basis.[1] Genetic factors are considered to be "the causal factors" for most of the conditions in the field of Medicine. Likewise, whatever be the etiology of amenorrhea, the "genetic basis" needs to be emphasized. Genetic factors could be single gene disorders/chromosomal, or multifactorial. Among them, mostly it is chromosomes and their abnormalities, contributing to the constitutional etiology of amenorrhea. The incidence of the chromosomal abnormality (CA) in live births is around 90 per 10,000. Included in the incidence are the numerical (monosomy/trisomy/mosaicism) as well as the structural (translocation/ isochromosome/ deletion/duplication/ring) CA.[3] The reported incidence of CA in primary amenorrhea is 20-40%. Therefore, nearly 60-80% of patients with PA

have normal karyotype 46,XX, accompanying afflicted gonads without follicular apparatus, hypoplastic ovaries, hypoplastic uterus, and certain alterations in the phenotype.[4] In India, only a few studies on amenorrhea and its genetic basis have been reported.[5] In view of the above, with the help of cytogenetic techniques, the present study was undertaken, to ascertain the presence of CA, as the genetic basis of amenorrhea.

Methods. Eight hundred and sixty-five women with PA (620) and SA (245) were referred to Division of Human Genetics, Department of Anatomy, St. John's Medical College, Bangalore, India for cytogenetic investigations and counseling from St. John's Medical College Hospital, other hospitals, nursing homes in and around Bangalore, India during the period 1973 to 2005. Patients were referred not only from Karnataka but also from other states like Andhra Pradesh, Tamil Nadu, and Kerala. They were selected on the criteria that they had no periods by age 14 in the absence of growth or development of secondary sexual characters, or no periods by age 16 regardless of the presence of normal growth and development with the appearance of the secondary sexual characters or absence of periods for a length of time equivalent to a total of at least 3 of the previous cycle intervals or 6 months of amenorrhea in a woman who has been menstruating. Their age ranged from 14-32 years. For each patient a detailed proforma and consent form were obtained. Cytogenetic investigations included modified leucocyte microculture method, Giemsa-Trypsin-Giemsa (GTG) banding techniques, photography, and karyotyping.

The Institutional Ethical Review Board has approved a part of the study as PhD topic for 200 patients. Moreover, at the time of referral, from each patient or guardian, consent was obtained that the information may be presented or discussed or published. The identity and confidentiality of the patient was of course, strictly maintained.

Results. The karyotype distribution has been tabulated for the entire sample size as well as for the PA/SA categories (**Tables 1-3**). It is seen (**Table 1**) that 202 individuals with amenorrhea have had CA, and a higher percentage (26.13%) was observed in PA. Irrespective of the classification, PA or SA, numerical CA seemed to be frequent. Also, in PA, the presence of 46,XY karyotype seemed to be prevalent (34.57%). The types of numerical CA has been considered and 45,X, the X monosomy condition, the typical Turner syndrome karyotype has been determined in nearly 53.5% of individuals with PA. In contrast, in SA, X mosaicism karyotype seemed to be prevalent. The abnormal cell lines ranged from less than 10-96%. Once again, the presence of Y cell line has been frequent in PA (11-96%) (**Table 2**). Isochromosome for the long arm of X [i(Xq)] in the pure or in mosaic forms has been noticed more, both in PA and SA. The striking features are the less frequently seen X structural anomalies, such as deletion/duplication/fragment/ring in PA compared with SA. Moreover, in SA, the X; autosomal anomaly has been observed; whereas in PA, we observed the presence of the reciprocal and robertsonian translocation involving chromosome 14 (**Table 3**). The long system of the determined breakpoints in the structural CA (**Table 3**) as per ISCN 2005, are listed in **Table 4**.

The variants are the chromosomal polymorphisms/ heteromorphisms. They have been included as the coincidental findings, since, they are known to have 'nil' significant effect in the phenotype of the PA (7) [46,XX,inv(9) (3); 46,XX,15ps+ (2); 46,XX,16qh+ (1); 46,XX,21ps+ (1)] / SA (5) [46,XX,inv(9) (1); 46,XX,15ps+ (2); 46,XX,9qh+ (1); 46,XX22ps+ (1)] individuals.

Discussion. It has been reported that PA is often associated with endocrine disorders, gonadal/somatic anomalies as well as with sex CA. However, only a few of the individuals with SA are referred for cytogenetic studies, since CA in SA is considered to be rare. The cause of SA is mostly cortical or hypothalamic, with less severely disturbed endocrine function.[6] In the present study, the incidence of CA in amenorrhea was found to be 23.35%. The reported incidence in the literature ranges from 13-32%,[6-9] and numerical X chromosomal abnormality is frequent. The average frequency of CA in women with PA is around 42%, and the observed range is 16-78%.[6-11] In PA, a number of sex CAs have been reported. Broadly, the CA in PA could be grouped as the X numerical; X structural and 46,XY female, the sex reversal condition. Their frequencies from the literature are: 45,X (40-50%); X mosaicism (25-36%); X structural (8%), and 46,XY female (16%).[6,10] In the present study, the respective frequency is 35.85% (38/106 - 45,X); 23.6% (25/106 - X mosaicism); 28.3% (30/106 - X structural), and the rest 12.3% [13/106 (47,XXX or 45,X/46,XY or 46,XX/46,XY)]. In PA case, 34.6% showed 46,XY female condition. Also in the present study, the frequently reported structural CA in X, has been observed: isochromosome/marker/ fragment/ring formation/deletion and duplication in Xp or Xq. Additional findings are the reciprocal and Robertsonian translocations.

In SA, the opined incidence of CA is 4-33%,[6,8] and the CA has been found to be in the decreasing range of normal karyotype/X mosaicism/X structural CA/47,XXX and 45,X conditions.[1] The actual reported incidence is: X mosaicism (60%), X structural CA (20%), and 45,X

Table 1 - Chromosomal abnormality in amenorrhea.

Karyotype	Total N = 865 (%)	Primary amenorrhea N = 620 (%)	Secondary amenorrhea N = 245 (%)
Normal (46,XX)	663 (76.65)	458 (73.87)	205 (83.67)
Chromosomal abnormality	202 (23.35)	162 (26.13)	40 (16.33)
Numerical	92 (45.54)	71 (43.83)	21 (52.5)
Structural	47 (23.27)	35 (21.6)	12 (30)
46,XY female	63 (31.19)	56 (34.57)	7 (17.5)

Table 2 - Numerical chromosomal abnormality in amenorrhea.

Karyotype	Primary amenorrhea (PA) N=71 (%)	Secondary amenorrhea (SA) N=21 (%)
45,X	38 (53.52)	1 (4.76)
PA: 45,X[25]/46,XX[75]; 45,X[93]/46,XX[7]; 45,X[92]/46,XX[8]; 45,X[4]/46,XX[96]; 45,X[10]/46,XX[90]; 45,X[12]/46,XX[82]; 45,X[40]/46,XX[60]; 45,X[50]/46,XX[50]; 45,X[96]/46,XX[4]; 45,X[4]/46,XX[96]; 45,X[7]/46,XX[93]; 45,X[10]/46,XX[90]; 45,X[92]/46,XX[8]; 45,X[50]/46,XX[50]; 45,X[8]/46,XX[92]; 45,X[42]/46,XX[58]; 45,X[67]/46,XX[33]; 45,X[20]/46,XX[80] SA: 45,X[30]/46,XX[70]; 45,X[87]/46,XX[13]; 45,X[8]/46,XX[92]; 45,X[3]/46,XX[97]; 45,X[7]/46,XX[93]; 45,X[4]/46,XX[96]; 45,X[9]/46,XX[91]; 45,X[15]/46,XX[85]; 45,X[66]/46,XX[34]; 45,X[3]/46,XX[97]; 45,X[10]/46,XX[90]; 45,X[12]/46,XX[88]; 45,X[35]/46,XX[65]; 45,X[27]/46,XX[73]	18 (25.35)	14 (66.66)
PA: 45,X[20]/47,XXX[20]/46,XX[60]; 45,X[7]/47,XXX[7]/46,XX[86] SA: 47,XXX[49]/45,X[19]/46,XX [32]; 45,X[9]/47,XXX[2]/46,XX[89]	2 (2.81)	2 (9.52)
PA: 45,X[80]/47,XXX[20] SA: 45,X[92]/47,XXX[8]	1 (1.4)	1 (4.76)
47,XXX[4]/46,XX[96]; 47,XXX[15]/46,XX[85]	2 (2.81)	-
47,XX+10[6]/46,XX[94]	1 (1.4)	-
47,XX+22[20]/48,XX+9+22[7]/46,XX[73]	1 (1.4)	-
47,XXX	1 (1.4)	-
PA: 45,X[89]/46,XY[11]; 46,XY[64]/45,X[36]; 46,XY[80]/45,X[20]; 45,X[60]/46,XY[40]; 46,XY[96]/45,X[4] SA: 45,X[90]/46,XY[10]; 46,XY[92]/45,X[8]	5 (7.04)	2 (9.5)
47,XYY[60]/45,X[40]	1 (1.4)	-
46,XX[98]/46,XY[2]	1 (1.4)	-
47,XXY[10]/46,XX[90]	-	1 (4.76)

Table 3 - Structural chromosomal abnormality in amenorrhea.

Karyotype	Primary amenorrhea (PA) N= 35 (%)	Secondary amenorrhea (SA) N = 12 (%)
46,X,i(Xq)	5 (14.29)	3 (25)
46,XX,t(9;14)(q22;q32)	1 (2.86)	-
45,XX,der(13;14)(q10;q10)	1 (2.86)	-
46,XX,t(X;12)(q23;q24)	-	1 (8.33)
46,XX,t(X;14)(q27;q24)	-	1 (8.33)
46,X+mar	1 (2.86)	-
46,XX, fra(16)(q22)	1 (2.86)	-
46,XX,del(9)(q12)[6]/46,XX[94]	1 (2.86)	-
PA: 46,X,del(X)(q22); 46,X,del(X)(q26) SA: 46,X,del(X)(q13)	2 (5.71)	1 (8.33)
46,X,dup(X)(q13q22)	1 (2.86)	-
45,X[55]/46,X+mar[45]; 46,X+mar[94]/45,X[6]; 45,X[88]/46,X+mar[12]	3 (8.57)	-
45,X[40]/46,X,del(X)(p22)[60]	1 (2.86)	-
45,X[18]/46,X,r(X)(p22.3q27)[82]	1 (2.86)	-
45,X(15p+)[60]/46,X,i(Xq)(15p+)[40]	1 (2.86)	-
45,X[60]/46,X,der(X)[40]*	1 (2.86)	-
PA: 46,X,i(Xq)[88]/45,X[12]; 46,X,i(Xq)[76]/45,X[24]; 45,X[82]/46,X,i(Xq)[18]; 45,X[94]/46,X,i(Xq)[6]; 46,X,i(Xq)[83]/45,X[17]; 46,X,i(Xq)[92]/45,X[8]; 46,X,i(Xq)[83]/45,X[17]; 46,X,i(Xq)[90]/45,X[10]; 45,X[98]/46,X,i(Xq)[2]; 46,X,i(Xq)[96]/45,X[4]; 45,X[64]/46,X,i(Xq)[36] SA: 46,X,i(Xq)[91]/45,X[9]; 46,X,i(Xq)[86]/45,X[14]; 45,X[93]/46,X,i(Xq)[7]; 45,X[59]/46,X,i(Xq)[41]	11 (31.42)	4 (33.33)
PA: 45,X[88]/46,X,del(X)(q24)[12]; 45,X[70]/46,X,del(X)(q22)[30] SA: 46,X,del(X)(q22)[93]/45,X[7]	2 (5.71)	1 (8.33)
45,X[65]/46,X+mar[35]	-	1 (8.33)
46,XX,Chrb(3)(p14)[10]/46,XX[90]	1 (2.86)	-
46,X,dup(X)(q12q13)[13]/46,XX[87]	1 (2.86)	-

Table 4 - The detailed system for the break points in structural chromosomal abnormality.

Primary amenorrhea
46,XX,t(9;14)(q22;q32)(9pter→9q22::14q32→14qter;14pter→4q32::9q22→9 qter)
45,XX,der(13;14)(q10;q10)(13qter→13q10::14q10→14qter)
46,XX,del(9)(q12)(pter→q12:)
46,X,del(X)(q22)(pter→q22:)
46,X,del(X)(q26)(pter→q26:)
46,X,dup(X)(q13q22)(pter→q22::q13→qter)
45,X/46,X,r(X)(p22.3q27)(::p22.3→q27::)
45,X/46,X,del(X)(p22)(qter→p22:)
45,X/46,X,del(X)(q24)(pter→q24:)
45,X/46,X,del(X)(q22)(pter→q22:)
46,X,dup(X)(q12q13)(pter→q13::q12→qter)
Secondary amenorrhea
46,XX,t(X;12)(q23;q24)(Xpter→Xq23::12q24→12qter; 12pter→12q24::Xq23→Xqter)
46,XX,t(X;14)(q27;q24)(Xpter→Xq27::14q24→14qter;14pter→14q24::Xq27→Xqter)
46,X,del(X)(q13)(pter→q13:)
46,X,del(X)(q22)(pter→q22:)

(20%).[6] In the present study, the frequencies are: 51.5% (X mosaicism); 36.4% (X structural); 3% (45,X), and the rest was 9.1% with 45,X/46,XY or 46,XX/47,XXY. Chromosomal abnormality in SA also has been more or less as noticed in literature, and 2 unusual cases of X; autosomal translocations have been detected in SA. In the present study, the CA, whether, numerical or structural, has occurred in decreasing order of frequency between PA and SA. Thus, the karyotype usually seen in PA is 45,X. These women may have Turner stigmata: short stature, webbing of neck, hypoplasia or aplasia, nipples far apart, barrel shaped chest, cubitus valgus, pigmented nevi, streak gonads, or hypoplastic uterus. The X chromosomal mosaicism could be a frequent finding in PA with or without gonadal dysgenesis, and analysis of multiple tissues may increase the chances of detecting the mosaicism. In mosaicism, the phenotype may depend on the percentage of normal cell lines with 46 chromosomes to abnormal cell lines with increase or decrease in the chromosome number especially X chromosome. Mosaicism is mostly associated with 45,X karyotype (45,X/46,XX; 45,X/46,XX/47,XXX; 45,X/46,XY).[4,6] Patients with 45,X/46,XY karyotype also may manifest a wide phenotypical variability. Many of them have shown the features of Turner syndrome, variable gonadal morphology ranging from a testis to a streak gonad.[6] The 46,XY could also be seen as a karyotype in PA. It is important to determine the karyotype, as the presence of Y chromosome is an indication for the surgical removal of gonads, which otherwise may lead to the risk of malignancy.[12] Structural abnormalities leading to PA mostly are isochromosome of the long arm of X [i(Xq)].[6] The structurally abnormal X chromosome may be inactivated thereby, may minimize the disturbance of cellular function.[13] Moreover, the phenotype may be indirectly influenced as per the size, and the loss/gain/altered genetic function, in the deleted or duplicated segments in X. In the translocations too, it has been opined, that the phenotype may be affected as per the chromosomes involved, including the regions/ breakpoints/size of the chromosomal segments and the position effect of the critical/ adjacent genes. The X–autosomal translocation has also been reported in patients with PA or SA. In X–autosomal translocation, the break point of the X is in the mid region of the long arm of X, then, it may lead to gonadal dysgenesis.[14-16] In the present study, X; autosomal translocations have been determined in SA, whereas, in PA, reciprocal and Robertsonian translocations have been observed. A note on the incidence of the translocations has to be added; reciprocal in 1/500 individuals, Robertsonian 1/1000 and X; autosomal 1/33,000. Such a rare finding of X; autosomal translocation has been determined in 2 secondary amenorrheic cases out of the 245 referral with SA.

In India, few studies have been reported in scientific programs rather than as publications on primary amenorrhea and its genetic basis. Sixty-three patients with PA have been studied and only 10 of them had CAs. The abnormalities involved the X chromosome (mosaicism, structural alterations-del/isochromosomes/ ring) resulting in the Turner syndrome phenotype.[5] In contrast to the prevalence of CA in patients with PA, as one of the etiological factors, the effect of a single gene on PA seemed to be negligible. In case of PA patients with 46,XX karyotype and gonadal dysgenesis and that too familial, then the autosomal recessive mode of inheritance has been suggested.[17] Mutations in the genes having direct or indirect influence especially on the neuro – endocrine systems associated to amenorrhea have also been stated.[1] The role of single gene mutations may be reflected from the hormonal assay, considered to be another essential investigation in the flow chart of detecting the etiology in amenorrhea. From the observations of the present study, it is apparent, that women with the absence of menstruation should be investigated for the determination of CA. The clinical signs in PA have also been noticed to be variable. Moreover, the phenotype/karyotype correlation may also become difficult, as a hidden mosaicism could never be ruled out in individuals with pure 45,X condition. Also, PA individuals, in spite of the presence of an apparently normal phenotype, should not be excluded from cytogenetic investigations. Even though for the SA, the presence of CA has been reported to be less, the observations of this study have shown that CA could be the etiology in significant proportions of women with SA. Any variation may be due to the sampling variation, the number, and the methodology. There is a chance in the total number of analyzed spreads for the X mosaicism and also given in the karyotype, as in general the percentage of abnormal cell line is less than 10% then it would have not been included in the mosaic category.[18]

At the time of counseling,[1,19] it should be informed that in women with sex chromosome anomalies, especially having X mosaicism, pregnancy cannot be ruled out. Nowadays, even in 45,X condition with hormonally prepared milieu, assisted reproductive strategy has become successful. The educational/ cultural/regional/religious, and the psychological background of the women with amenorrhea and their family should be kept in mind. Also, during counseling the following information also ought to be provided: mechanism of origin of the chromosomal anomalies, the almost negligible recurrence risk in sex chromosomal anomalies, the hormonal therapy, education/hobby/ career, marriage/reproductive options.

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