Original Article

Chromosomal Abnormalities in Amenorrhea: A Retrospective Study and Review of 637 Patients in South India

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Abstract

Background: The aim of the present study was to investigate the chromosomal abnormalities and to identify the most prevalent or frequent type of chromosomal abnormalities in cases of amenorrhea from the southern region of India.

Methods: A total of 637 cases with amenorrhea were analyzed using G- banding, C-banding, Silver staining, and fluorescence *in situ* hybridization was done wherever necessary.

Results: Out of the 637 cases involved in our study, 132 abnormalities were detected. The incidence of chromosomal abnormalities in cases with primary and secondary amenorrhea was around 20.7 %. In addition to the numerical anomalies, various structural aberrations of the X chromosome like deletions, isochromosomes, duplications, ring chromosome, and also male karyotype were detected.

Conclusion: Review of the literature and overall incidence of chromosomal abnormalities in patients with amenorrhea suggests the need for cytogenetic analysis to be performed in all the cases referred for amenorrhea with or without short stature. Precise identification of chromosomal abnormalities helps in confirming the provisional diagnosis; it helps the secondary amenorrhea patients in assisted reproduction and to understand the clinical heterogeneity involved and in efficient genetic counseling.

Keywords: Chromosomal abnormalities, karyotype, primary amenorrhea, secondary amenorrhea

Cite this article as: 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(5):** 267 – 270.

Introduction

Amenorrhea means absence of menstruation during puberty or later in life. Amenorrhea is not a disease but it is a symptom that results due to several different causes. These causes include pregnancy, absence of uterus and vagina, hormonal imbalance, excess of male testosterone, endometritis, improper functioning of ovaries,[1,2] and Mullerian agenesis.[3] The referral cases which fall into these categories are considered as amenorrhea cases. There are two types of amenorrhea; they are primary amenorrhea (PA) and secondary amenorrhea (SA). The first category i.e., PA is defined in two different groups. In the first group, it is defined as the absence of menarche by age 14 years, with no development of secondary sexual characters. In the second group, it is defined as the absence of menarche by age 16 years with normal development of secondary sexual characters. The second category i.e., SA is defined when there is one or more bleeding episodes followed by a minimum three months of amenorrhea.[4] The other causes of SA may be due to polycystic ovaries, gonadal dysgenesis, or due to pregnancy. Incidentally, it is also present in patients with X/ autosomal translocations.[5]

WHO had estimated that out of the 15 % infertility in the human population, amenorrhea stands as sixth largest major cause of female infertility. Additionally, among general population amenorrhea affects 2% – 5 % of all women in the child- bearing age. Also, genetic factors like single gene disorders/, chromosomal, or multifactorial disorders are also mentioned in the literature. Among them it is mostly chromosomal abnormalities which contribute to the constitutional etiology of amenorrhea.[6] Cytogenetic investigations have shown the importance of chromosomal abnormalities as a major cause of amenorrhea.[6,7] Many amenorrhea patients show a variety of chromosomal abnormalities like 45,X and sex reversals i.e., female phenotype with male chromosomal compliments and also other autosomal translocations. In fact, all around the world contribution of sex chromosomal abnormalities to the problem of PA is well documented. These sex chromosomal abnormalities may be numerical with XO/XXX and structural showing Xp/q duplications or deletions, ring chromosomes, isochromosomes, and also mosaics leading to PA.[8]

In the literature it is described that the percentage of chromosomal abnormalities varies from 15.9% to 63.3 % in PA[2,9] and 3.8% to 44.4 % in SA.[2,10] This wide variation is due to different sample size of selection group. Our study aimed to determine the frequency of chromosomal abnormalities and also to find the most prevalent chromosomal anomalies present in PA and SA in the southern region of India.

Materials and Methods

A retrospective study was carried from the period February 1998 through February 2012. A total of 637 cases referred for amenorrhea were offered chromosomal analysis. In all the cases the detailed case histories and written consent were taken and karyotypes were generated from the peripheral blood lymphocyte cultures and the cytogenetic analysis was performed. Out of the 637 cases, 251 were for suspected PA, 28 with SA, and 358 cases with other reasons like stunted growth, features of Turner syndrome

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Accepted for publication: 23 January 2013

(TS), etc. The age group varied from eight years to 38 years. The different referral reasons included PA, SA, features of TS, stunted growth, androgen insensitivity syndrome, absent vagina, absent uterus, hypoplastic uterus, and oligoamenorrhea.

Cytogenetic study

Metaphase chromosome preparations from peripheral blood were made according to the standard cytogenetic protocols. Chromosomal analyses were performed by G-banding using trypsin and Giemsa (GTG) at approximately 400 – 450 band level. Further nucleolar organizing regions (NOR) staining and C-banding were also performed wherever required for confirming satellites and heterochromatic region.

Twenty metaphases were analyzed in all the patients, but in cases of abnormalities and mosaicism the study was extended up to 50 metaphases. All the chromosomal abnormalities were reported according to the current international standard nomenclature (ISCN, 2009).

Results

A total of 637 cases were evaluated for both PA and SA, out of which 132 cases showed chromosomal abnormalities. The total chromosomal abnormalities identified were 20.7%. Chromosomal abnormalities in our study are broadly classified into numerical (55), structural (41), and other abnormalities (36) with 505 cases showing normal female complementation. The details of the karyotypes and percentage of abnormalities of all the 637 cases, along with the cytogenetic grade are given in detail in Table 1.

In our study, we had classified chromosomal abnormalities into five main types with or without mosaicism. They are:

a) Numerical anomalies: The most frequent karyotype was X chromosome aneuploidy. These include 45, X (48), 47, XXX (2), and mosaic Turner with numerical aberrations (5).
b) Male karyotype: Found in 13 cases and mosaic 45, X/46, XY in four cases.
c) Pure structural X chromosomal abnormalities: Seven patients were found to have isochromosome of long arm of X chromosome, four patients with short arm, and three patients showed deletions on X chromosome. Mosaic Turner with structural aberrations of X: Ring chromosome (4), isochromosome (17), deletion (4), and duplication (2).
d) Autosomal structural anomalies: Five cases were found to have autosomal –autosomal translocations.
e) Variants: Eleven of the referred cases showed common variants exhibited in the general population and three cases of Turner also showed variants.
f) The ratio of the cytogenetic finding of PA with SA was around 10:3.

Table 1. Karyotype of the patients with amenorrhea showing the number of cases

No	Cytogenetic grade	Karyotype	No. of cases	%
1	Normal	46, XX	505	79.3
2	Pure Turner	45, X0	48	7.5
3	Pure X numerical abnormalities	47, XXX	2	0.3
4	Pure structural X chromosomal abnormalities	46, X, i(Xq10) 46, X, i(Xp10) 46, X del(X)(q21.2→qter) 46, X del (X)(q22.3→qter)	5 4 1 2	0.8 0.6 0.2 0.3
5	Mosaic Turner with numerical aberrations of X	45, X/46, XX 46, XX/47, XXX 45, X/47, XXX 46, XX/47, XX+21	2 1 1 1	0.3 0.2 0.2 0.2
6	Mosaic Turner with structural abnormalities	45, X/46, Xr(X)(p22q22) 45, X/46, Xi(Xq10) 45, X/47, XXp- 45, X/46, Xdel(X) 45, X/46, Xi(Xp10) 45, X/46, X, delX(q21.2;qter) 45, X/46, X, del(X)q25-qter 45, X/46, X, dup(X)p22.1-pter 45, X/46, Xdup(X)(q12q21) 45, X/46, X, i(Xp10)/46,XX	4 12 1 1 6 1 1 1 1 1	0.6 1.9 0.2 0.2 0.9 0.2 0.2 0.2 0.2 0.2
7	Pure XY females	46,XY	13	2.0
8	Variants	46, XX, inv(9) 46, XX, 1qh+ 46, XX+22ps+ 46, XX+14ps+ 46, XX+15ps+ 46, XX+9qh+	3 4 1 1 1 1	0.5 0.6 0.2 0.2 0.2 0.2
9	Turners with variants	45, X, i(X),15ps+ 45, X,14ps+	2 1	0.3 0.2
10	Autosomal structural abnormalities	46, XX, t(3; 9)(q29; q32) 46, XX, t(10; 12)(p15; q21)mat 46, XX, t(2; 18)(q31; p11.2) 46, XX, t(14; 19) 45, XX, t(13; 14) /46, XX, t(13; 14)+X	1 1 1 1 1	0.2 0.2 0.2 0.2 0.2
11	Mosaic XY females	45, X/46, XY	4	0.6

Table 2. Review of the literature in comparison with the present study

Author (year)	Total cases	X chromosome aneuploidy	Structural anomalies	Marker chromosome	Male karyotype	% abnormal chromosome
Opitz, et al. (1983)[2]	88	17%	7%	---	4%	28%
Ten, et al. (1990)[9]	117	8%	7%	2%	14%	31%
Wong, et al. (2005)[11]	237	12.6%	2.9%	0.4%	8.4%	24.5%
Rajangam, et al. (2006)[6]	865	45.54%	23.27%	---	31.19%	23.35%
Safaei, et al. (2010)[20]	220	10.9%	3.6%	---	6.4%	20%
Kalavathi (2010)[21]	979	10.41%	6.90%	---	6.23%	23.39%
Laxmi, et al. (1997)[22]	70	25.7%	---	---	2.8%	28.5%
Roy, et al. (1995)[23]	60	60%	---	---	3.3%	63.3%
Anglani, et al. (1984)[24]	145	25%	10%	---	11%	46%
Joseph & Thomas (1982)[25]	63	8%	3%	2%	3%	16%
Vijayalaksmi, et al. (2010)[26]	140	14.28%	7.14%	---	6.42%	27.8%
Our cases (2011)	**600**	**8.7%**	**9.2%**	---	**3%**	**21 %**

Conclusion

Many surveys have been undertaken worldwide to ascertain the frequency of chromosomal anomalies in patients with primary and SA. Apparently, majority of them showed small sample size. But in this study, we could recruit a large number of patients thus increasing the sample size. However, most of the cases in this study were referred for PA than SA.

A review of the literature shows that chromosome anomalies appear to be one of the main causes of PA. The reported percentage of chromosomal abnormalities varies greatly from 15.9 % to 63.3 %. This wide variation in the percentage is due to the variation in the sample size and also patient selection criteria. The estimated frequency in our study was 20.7 % which is thus comparable with the chromosomal abnormalities described in the literature. Review of the literature for amenorrhea cases is compared with our present study and presented in Table 2. Further, most of the described cases are sex chromosomal aberrations with numerical and structural variations. In most of the rural parts of India, majority of the patients with sex- related anomalies do not visit a clinic for undergoing any tests; thus, the actual frequency of sex chromosome abnormalities in patients with amenorrhea remains undetermined.

In almost all the studies reviewed, the most frequent chromosomal anomaly in amenorrhea patients is 45, X followed by a male karyotype.[11] The phenotypic features may vary in all these patients but the physical presentation of the male karyotype may occur later on. In our study also the frequency of occurrence of 45, X was the highest followed by male karyotype.

As the causes of amenorrhea vary, many investigators have suggested polycystic ovarian syndrome (POS) to be one of the most frequent causes of SA which may be genetically determined. Some reports have also shown chromosome alterations in a few patients with POS.[12] But POS may be secondary to X chromosome deletions or translocations. Reports with POS and Xq deletions suggest that there is a gene (POF1) localized to Xq21.3-q27 or within Xq26.1-q27[13,14] and a gene (POF2) localized to Xq13-q21.1.[15] Fraccaro, et al.[16] showed that Xp21→pter region loss is compatible with fertility, probably because it leaves on Xp, the region which is never inactivated. In this study, most patients showed the breakpoint regions, comparable with the suggested POF genes. Two patients had an Xp21 deletion, indicating that the short arm is also crucial for fertility. Triple X also have high rate of ovarian failure. Thus, patients with SA should be referred for cytogenetic analysis to rule out any chromosomal abnormalities.

In TS cases, the genes involved in gonadal function are located on the proximal part of Xp and also on the distal part of the Xq, where as the genes whose absence is responsible for somatic features of the syndrome may be distributed along the length of Xp and the middle section of Xq (q21-q26).[17] Apart from genes on sex chromosomes, there are also few autosomal genes involved in short stature like SHOT, which is a SHOX homologous gene on chromosome three. For example, the unique case which we described in our study involving chromosomes 14 and 19 with short stature in a girl may help us in the identification of some new genes present on autosomes. Hence, molecular characterization of such rare cases with similar phenotypes helps in the identification of new genes which are involved in short stature.

Considering the data from the literature as well as our own findings, the suspicion of a sex chromosome anomaly is in 1) patients with PA, 2) patients with SA, 3) patients who, besides amenorrhea, present other clinical signs suggestive of TS.[18] Identification of such chromosomal abnormalities at an early stage helps in surgery, counseling, and if mosaic to state the reproductive stages and premenopausal details.[19] In our study, the patients with pure monosomy X were invariably infertile. But recently even in such patients hormonally prepared milieu, assisted reproduction strategy had become successful.

In conclusion, the results of the percentage of chromosomal abnormalities found in our samples are consistent with figures described in sample sizes in the literature. The overall percentage of chromosomal abnormalities indicates that the chromosomal analysis be performed in all cases of amenorrhea essentially with features of Turner's like, short stature, etc at an early stage. Early identification of such chromosomal abnormalities especially in cases of SA helps in assisted reproduction and also decides about further reproductive options; in mosaic cases the chances of fertility can be predicted. Ascertainment of the karyotyping aids in confirmation of the provisional diagnosis, a better phenotype-genotype correlation to understand clinical heterogeneity, and in genetic counseling. Genetic counseling should include the risk of gonadal malignancy for patients with XY gonadal dysgenesis, the risk of premature menopause for patients with TS and the use of hormonal replacement therapy, and the possibility of infertility in the future children of patients with mosaic TS cases.

Acknowledgments

This study was supported by a core grant to the Center for DNA Fingerprinting and Diagnostics, from the Department of Biotechnology, Government of India.

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