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Original Article

Cytogenetic Analysis of Patients with Primary Amenorrhea in Southwest of Iran

Akbar Safaei[1], Mohammad Vasei[2], Hossein Ayatollahi[1,3]

1. Dept. of Pathology, Shiraz University of Medical Sciences, Shiraz, Iran
2. Dept. of Pathology, Tehran University of Medical Sciences, Tehran, Iran
3. Dept. of Pathology, Mashhad University of Medical Sciences, Mashhad, Iran

ABSTRACT

Background and Objectives: **Primary amenorrhea is not a disease but a symptom that may result from several quite different causes. Common hormonal cause of primary amenorrhea includes constitutional delay, hypothalamic –pituitary dysfunction, chronic systemic disease and absent ovarian function. The aim of this study was to estimate the incidence of the chromosomal abnormality referred for karyotyping in patients with primary amenorrhea in southwest of Iran.**

Material and Methods: **Chromosomal analysis was carried out in 220 such cases that were referred from different parts of the south of Iran. The standard protocol for peripheral blood lymphocyte culture was followed for metaphase chromosome preparation and conventional analysis of G-banded chromosome.**

Results: **The frequency of abnormal karyotypes was 20% in primary amenorrhea. The chromosomal abnormalities can be classified into five main types with or without mosaicism. 1-The most frequent karyotype was X chromosome aneuploidies (10%,n=22) 2-Male karyotype 46, XY was present in 5.5 % (n=12). 3-Structural anomalies of the X chromosome were detected in 3.2% (n=7) . 4-Mosaicism of male chromosome constitution and X chromosome aneuploidy was present in two (0.9%) cases (45XO/46XY). 5-Mosaicism of X chromosome aneuploidy and structural anomalies of X chromosome was found in one (0.45%) case [45, X/46X, i (Xq)].**

Conclusion: **The present study has emphasized that karyotyping is necessary in evaluation of primary amenorrhea. This study also revealed the incidence of chromosomal abnormalities in women with primary amenorrhea in southwest of Iran is similar to that reported in previous literatures.**

Keyword: Primary Amenorrhea, Cytogenetic Study, karyotyping, Iran

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Address communications to: Dr Hossein Ayatollahi, Department of Pathology, Mashhad University of Medical Sciences, Mashhad, Ira
Email: Ayatollahih@mums.ac.ir

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Introduction

Primary amenorrhea is defined as the absence of menstruation and secondary sexual characteristics in phenotypic women aged 14 years or older or aged 16 or older if secondary sexual characteristics were present (1). There are many reasons for primary amenorrhea but genetic or chromosomal abnormality is the most important causes and presence of chromosomal abnormality affects subsequent management (2).

Hormonal disorders are main causes of primary amenorrhea, although there are others.

Common hormonal cause of primary amenorrhea includes constitutional delay, hypothalamic –pituitary dysfunction, chronic systemic disease and absent ovarian function.

Cytogenetic investigation has shown the importance of chromosomal abnormalities as a cause of amenorrhea (3-6).

Some patients with primary amenorrhea may have chromosomal abnormalities, or are cases of sex reversal, i.e. patients with female phenotype but with normal male chromosome complement. The sex chromosome abnormalities may be numerical, as XO patients or structural, with patients having abnormally small X chromosome due to deletion or abnormally large X chromosome and some type of mosaicism of the X chromosome such as XO/XXX and XO/XX can also lead to primary amenorrhea (7-9).

A number of surveys in various parts of the world have endeavored to ascertain the contribution of sex chromosome abnormalities to the problem of primary amenorrhea.

The percentage of chromosomal abnormalities reported varies greatly, from 15.9% to 63.3% for primary amenorrhea (10-16). The wide variation is likely due to different selection criteria of different studies.

The aim of this study was to present the cytogenetic findings in patients with primary amenorrhea in Southwest of Iran.

Material and Methods

In this cross sectional study, all women with primary amenorrhea who were referred to the Cytogenenetic Ward of Department of Pathology –Shiraz University of Medical Sciences-Iran from 1 January 2005 to 30 March 2008 were recruited.

Primary amenorrhea was defined as the absence of menstruation and secondary sexual characteristics in phenotypic women aged 14 years or older or aged 16 or older if secondary sexual characteristics were present.

The diagnosis of primary amenorrhea was ascertained at the patient's initial visit and physical examination was performed to identify any secondary sexual characteristics or syndrome feature.

Laboratory investigation and clinical information were obtained from hospital records or the referring physician.

Their age at presentation ranged from 14 to 38 years with a mean of 20.62±4.12years.

All patients gave informed voluntary consent to participate in the study according to the protocol approved by the local Ethics Committee of SUMS and in accordance with the ethical standards of the Helsinki Declaration. For all chromosome investigations of routinely cultured lymphocyte, we used G-banding (17).

Briefly, cultures of peripheral blood lymphocytes in RPMI 1640 basal medium and 10% fetal calf serum (Gibco-Invitrogen-USA) were treated with 0.1 microgram/ml of colcemid (Gibco-Invitrogen-USA) after a 72–h incubation period and then methaphase chromosomes were spread and stained using standard G-banding technique.

For each case, 15 metaphase spreads were analyzed with Cytovision Chromosomal Karyotyping Automatic system (Genetix Company-USA) and when mosaicism was suspected, at least 50 metaphases were examined.

Results

There were 220 women referred for primary amenorrhea during the study period. Age at referral to our center ranged from 14 to 38 years with a mean of 20.62±4.12 years. The frequency of abnormal karyotypes was 20% in primary amenorrhea.

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The chromosomal abnormalities can be classified into five main types with or without mosaicism:

1-The most frequent karyotype was X chromosome aneuploidies (10%,n=22) these include Turner syndrome 45,X (n=19),mosaic Turner , 45,X/46,XX(no=2) and 45,X/47,XXX(no=1). The percentage of mosaicism ranged from 10% to 70%.

2-Male karyotype 46, XY was present in 5.5 %(n=12).

3-Structural anomalies of the X chromosome were detected in 3.2%(n=7) .Four patients were found to have isochromosome of long arm of X chromosome [46X,i(Xq)](Figure-1),one patient has isochromosome of short arm of X chromosome [46X,i(Xp)],one patient has partial deletion of X chromosome and one patient has X-autosome translocation[46,XX,t(X;3)].

4-Mosaicism of male chromosome constitution and X chromosome aneuploidy was present in two (0.9%) cases (45XO/46XY).

5-Mosaicism of X chromosome aneuploidy and structural anomalies of X chromosome was found in one (0.45%) case [45,X/46X,i(Xq)] .

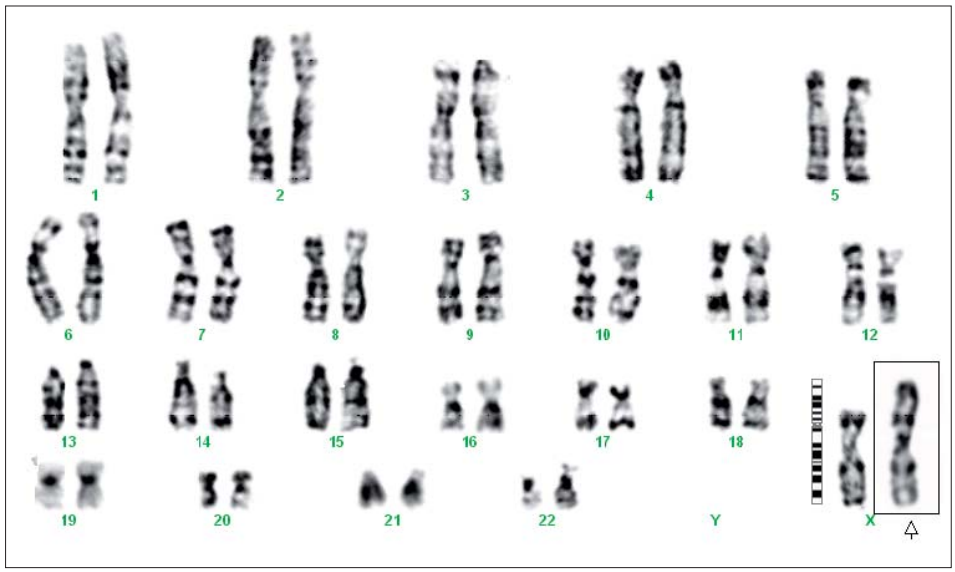

Fig. 1:G-banded karyotype of patient with isochromosome X

Discussion

A large number of survey have been undertaken worldwide to ascertain the frequency of chromosomal anomalies in patients who present with primary amenorrhea.

Distribution of chromosomal abnormalities in primary amenorrhea cases based on numerical and structural anomalies in 29 studies from 1961 to 1992 was given by Lakshmi et al (18).

According to this study, there were altogether 2156 patients with primary amenorrhea studies from all the 29 series, out of which 697 patients exhibited chromosomal abnormalities (32.32%). Further ,out of these 697 patients with chromosomal abnormalities, 663 patients showed numerical abnormality (95.12%) and remaining 34 patients revealed structural abnormalities(4.88%).It is also seen from this report that the chromosomal abnormalities range from 6.66% - 56.22% in various study. The upper limit of 56.22% was reported by Barucha et al (19) in 1992 and lower limit of 6.66% was reported by Joseph et al. (13) in 1989.

In another study by Wong et al (20) previous estimates of the frequency of sex chromosomal abnormalities vary from 15.9% to 63.3% for primary amenorrhea, with the majority falling between 20% and 30% (Table 1).

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Table 1: Chromosome abnormalities in primary amenorrhea in various study and compared with present study

	Present study	Wong *et al.* (21)	Temocin *et al.* (12)	Roy *et al.* (14)	Ten *et al.* (15)	Van Niekerk *et al.* (15)	Series compiled before 1978(15)
Study population	Iran	Hong Kong	Turkey	India	Malaysia	South Africa	-
No .of case	220	237	68	60	117	77	336
46,XX	176(80%)	179(75.5%)	50(73.5%)	22(36.5%)	81(69.2%)	56(72.7%)	239(71.1%)
Abnormal karyotype	44(20%)	58(24.5%)	18(26.5%)	38(63.3%)	36(30.8%)	21(27.3%)	97(28.9%)
46XY	12(5.5%)	20(8.4%)	-	2(3.3%)	16(13.7%)	4(5.2%)	29(8.6%)
45X	19(8.6%)	14(5.9%)	-	16(26.7%)	9(7.7%)	7(9.1%)	39(11.6%)
Mosaic 45,X	3(1.4%)	15(6.3%)	-	20(33.3%)	-	8(10.4%)	23(6.8%)
46X,del(X)	1(0.45%)	4(1.7%)	-	-	-	-	-
46X,i(Xq)	4(1.8%)	1(0.4%)	-	-	-	-	-
46X,i(Xp)	1(0.45%)	-	-	-	-	-	-
X-A Translocation	1(0.45%)	2(0.8%)	-	-	-	-	-
Mosaic triple X	-	1(0.4%)	-	-	-	-	-
46,X,+ mar	-	1(0.4%)	-	-	2(1.7%)	-	-
45X/46XY	2(0.9%)	-	-	-	-	-	-
45,XO/ 46X,i(Xq)	1(0.45%)	-	-	-	-	-	-

The estimated frequency following our study of 20% is thus comparable and the most frequent chromosomal abnormalities in our patients is Turner syndrome (8.6%) followed by patients with male karyotype (5.5%)

Male karyotype presented in a significant percentage of patients with primary amenorrhea in previous studies from 3.3% to 13.7% (Table 1) and our study is comparable with these studies.

In our study there was two new categories from combination another category for karyotype anomalies which described previously that include mosaicism of male chromosome constitution and X chromosome aneuploidy and another one is mosaicism of X chromosome aneuploidy and structural anomalies of X chromosome .

Conclusion

A significant number of patients had sex chromosomal abnormalities, thus early cytogenetic investigation is prudent to guide further management.

After exclusion of non-genetic causes by physicians and gynecologists, patients with primary amenorrhea, should receive prompt referral for genetic study.

Genetic counseling should include the risk of premature menopause for patients with Turner's syndrome and the use of hormonal replacement therapy, the risk of gonadal malignancy for patients with XY gonadal dysgenesis and the possibility of infertility in the future children of patients with mosaic Turner.

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