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Investigation of the Frequency and Type of Chromosomal Abnormalities in Women Patients with Amenorrhea

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Abstract

Background: Amenorrhea is defined as the absence of menstruation at the reproductive age of women. Amenorrhea caused by various etiological factors including genetic factors, intrauterine malformations, endocrine dysfunction, and environmental factors. Genetic factors particularly chromosomal abnormalities are the main cause of Amenorrhea. This study was performed to estimate the frequency and types of chromosomal abnormalities in patients with amenorrhea in the northeast of Iran.

Methods: A total of 381 women with the history of amenorrhea participated in this study. Peripheral blood lymphocyte cultures were performed according to the standard GTG banding method.

Results:296 (77%) of a total of all cases had a normal karyotype (46, XX) while 85 patients (23%) had abnormal karyotype. The numerical and structural abnormalities of X chromosome were observed in 52 (61%), the abnormalities of Y chromosome were observed in 23 (27.2%) and rearrangements between autosomal and/or sex chromosomes were observed in 10 (11.8%).

Conclusions: The present study revealed that cytogenetic study is essential for early diagnosis and treatments of Amenorrhea.

Keywords: Amenorrhea, Chromosomal Abnormalities, Cytogenetics.

Introduction

Amenorrhea is an unusual condition in a woman of reproductive years defined as the absence of menstruation during puberty or later in life (1). Two main classifications of amenorrhea are categorized according to the occurrence of menarche, Primary Amenorrhea (PA) and Secondary Amenorrhea (SA) (2). PA refers to lack of menstruation and secondary sexual traits in women at age 14 or normal secondary sexual traits at the age of 16 (3), SA is defined as when patients had one or more spontaneous bleeding episode, followed by the cessation of regular menses for at least 12 months before the age of 40 (4). Amenorrhea is a symptom with various causes. Amenorrhea may result from developmental disorders like the congenital missing of the uterus and defect of the ovary to receive or maintain egg cells. The prime causes of amenorrhea include chromosomal or genetic disorders that can lead to the ovaries stop functioning normally, hypothalamic, or pituitary disorders in the brain, and physical problems such as reproductive organs diseases that can stop menstruation from starting and extreme

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physical or psychological stress, or a mixture of these elements that can postpone the onset of menstruation (5). World Health Organization has estimated, amenorrhea is the sixth-largest major cause in women infertility. Moreover, amenorrhea affects about 2–5% of all women in the childbearing years in the general population (6). Amenorrhea is mainly caused by pituitary/hypothalamic dysfunctions (27.8%); gonadal dysfunction (50.4%) and outflow tract defect (21.8%) (7). The causes of amenorrhea can be classified as disorder of the hypothalamus, pituitary and uterus or ovaries including functional or anatomical problems, and genetic disorders which may be caused by chromosomal structure or gene mutation (7,8). Cytogenetic studies have revealed that a major cause of amenorrhea and then reproductive problems is a chromosomal abnormality which play a vital role in a further management (9). It has been shown that the percentage of chromosomal abnormalities differ from 15.9% to 63.3% in women with amenorrhea (7). These differences may be due to the frequency of the chromosomal disorder in a selected population or the limitation of participants in the study. The aim of this study was to establish the frequency and identification of chromosomal abnormalities among women with amenorrhea for the subsequent management and counseling.

Materials and Methods

Sample Collection

The participants of the study were 381 women 14-40 years old. The average age was 20.4 years who were referred to the Pardis Clinical and Genetics laboratory, Mashhad, Iran from 2013 until 2021. Patients with the symptoms of absence or irregular menstruation who were referred to a gynecologist and diagnosed as amenorrhea based on medical history, clinical information, hormonal profile, and ultrasonography were included in this survey. Patients with other clinical symptoms such as infertility and miscarriage were excluded. About 1-2 ml of heparinized peripheral venous blood sample was collected in sterile tubes from each patient and labeled with the specific patient non-identifier code.

Ethical approval

All patients provided their written informed consent for participation. The study was approved by the Institutional Review Board of Mashhad University of Medical Sciences, Mashhad, Iran (approval code: 951565).

Setting up of lymphocyte cultures

Lymphocyte cultures were performed according to the standard procedure with some modifications (10). Culture media were prepared by adding RPMI 1640, L-glutamine, (Gibco, Life Technologies, UK), 20% fetal bovine serum (Gibco, Life Technologies, UK), Penicillin–Streptomycin, and Phytohemagglutinin for stimulation of lymphocytes (Gibco, Life Technologies, UK). Approximately 0.5 ml of peripheral blood inoculated into a sterile tube with 5 ml of RPMI medium. Parallel cultures were made for each sample. The culture was Incubated for 72 hours. After Adding 0.2 ml of Colcemid Solution (Gibco, Life Technologies, UK) to each culture tube, the cultures were Incubated for 15 minutes additionally, colcemid were removed by centrifugation tube at 480 RCF for 10 minutes, the supernatant were removed, then added 10 ml of hypotonic solution (0.075 M KCl, Merck, Germany) incubated for 10 minutes. Spin at 480 RCF for 10 minutes. The supernatant was removed, agitated the cellular sediment, and added 5-10 ml of fresh, ice-cold fixative made up of one-part glacial acetic acid to three parts absolute methanol.

Slide Preparation and Karyotyping

Cell pellet were dropped onto a clean slide and allow air-drying at room temperature. The prepared slides were stained with GTG standard banding method. Initially, 15 metaphase spreads were analyzed for each case with Cytovision Chromosomal Karyotyping Automatic system (Genetix Company-USA).

In the case of mosaicism, minimum of 50 metaphases were analyzed. Karyotype results

were reported according to the International System for Human Cytogenetic Nomenclature (ISCN) recommendations, 2016.

Results

A total of 381 women were evaluated for chromosome abnormalities. Of the total number of cases with amenorrhea, 296 cases (77%) had a normal karyotype (46,XX) while 85 patients (23%) had abnormal karyotype. The details of the karyotype and frequency of abnormalities of all cases are listed in the Table 1.

Table 1. Karyotype and frequency of the patients with amenorrhea.

Cytogenetic category	Karyotyping	No. of cases (%)
Normal Abnormal karyotypes	46,XX Numerical and structural abnormalities	**296 (77%)** **85 (23%)**
1. Abnormalities of Chromosome X:		52 (61%)
A) Pure Turner	45, X	**29 (34.4%)**
B. Structural abnormalities:		**9 (10.2)**
I) Isochromosome Xq	46, X,i (Xq)	**4 (4.6%)**
II) Deletion Xq	46, X,del (X) (q12)	**3 (3.4%)**
III) Deletion Xp	46, X,del (X) (p22.1)	**2 (2.2%)**
C. Mosaicism of X:		**14 (16.4%)**
I)	45, X/46,XX,del (X) (p11.2)	**2 (2.3%)**
II)	45, X/46, X,i (Xq)	**7 (8.3%)**
III)	45, X/47XXX	**3 (3.5%)**
IV)	45, X/46,XX,del (X) (Xq22.1)	**2 (2.3%)**
2. Male Karyotype/		
Y chromosome Abnormalities		**23 (27.2%)**
I) pure XY	46, XY	**17 (20.1%)**
II) Mosaicism of XY	45,X/45,XY	**6 (7.1%)**
3. Women with autosomal/ sex chromosome structural abnormalities		**10 (11.8%)**
I)	46,XX,inv (9) (p11,q12)	**4 (4.7%)**
II)	45,XX,rob (13,14) (q10,q10)	**3 (3.5%)**
III)	46,XX,t (13,17) (p21,q25)	**1 (1.2%)**
IV)	46,XX,t (X,1) (q13,q24)	**1 (1.2%)**
V)	44,X,t (15,21)/45,X,i (xq)t (15,21)	**1 (1.2%)**

We have classified chromosome abnormalities into three main categories with or without mosaicism. The first category was the numerical and structural abnormalities of X chromosome 52 (61%), which was the most frequent abnormalities of X chromosome aneuploidy 29 (34%) (Fig. 1), followed by mosaic turner and structural abnormalities 14 (16.4%). The number of women who carried an Isochromosome Xq was 4 (4.6%). The numbers of deletion of long arm of chromosome X and short arm was 3 (3.4%) and 2 (2.2%) respectively. The second category was abnormalities of Y chromosome 23 (27.2%), with two subgroups pure XY chromosome 17 (20.1%) and mosaicism of XY chromosome 6 (7.1%). The third category was women carrying rearrangements between autosomal and/or sex chromosomes 10 (11.8%). We observed 2 cases of the translocations [46,XX,t (13;17) (p21;q25) and 46,XX,t (X; 1) (q13;q24)] in this category.

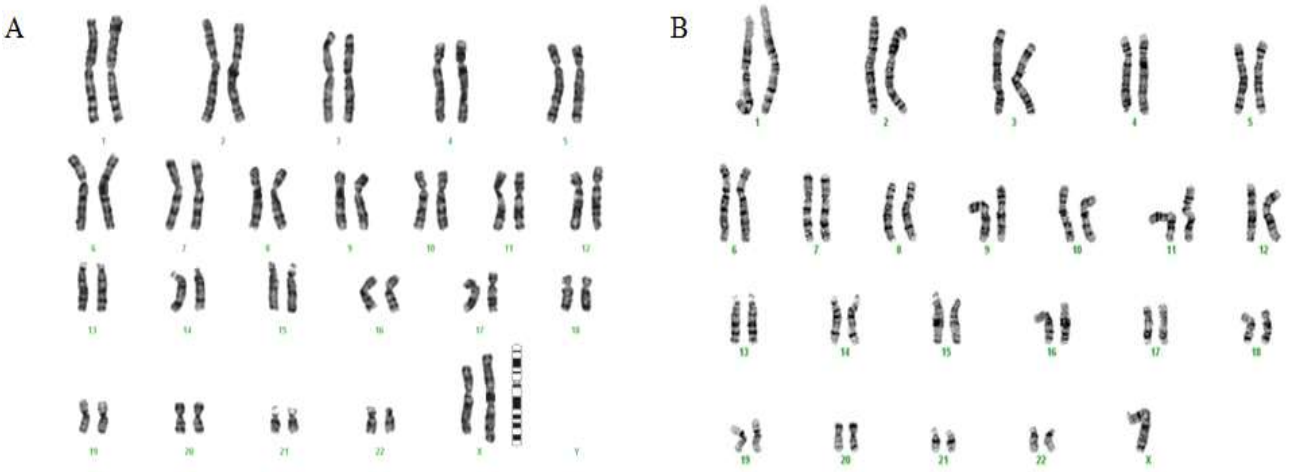

Fig. 1. (A) Karyotype (46,X,i (Xq)) of a primary amenorrhea woman with isochromosome Xq. (B) Karyotype (45, X) of a primary amenorrhea woman with Turner's syndrome.

Discussion

Amenorrhea is one of the main causes of women infertility affecting 2–5% of all women of child bearing years (6). Variety of reasons can cause Amenorrhea, it has been shown that approximately 40% of cases have genetic disorders (7). Single gene disorders or chromosomal abnormalities can contribute to the reasons for Amenorrhea. However, Cytogenetic studies have revealed that a major cause of amenorrhea and then reproductive problems in women is a chromosomal abnormality which plays a vital role in a further management (11).

Several studies have been conducted to determine the frequency of chromosomal abnormalities among patients with Amenorrhea. The frequency of abnormal karyotypes have been reported to vary between 16% and 63.3% among women with Amenorrhea in different parts of the world (11). These variations are mainly due to the different criteria of selecting patients and the small sample size.

In the present study, the karyotype results were normal in 296 (77%) cases. The frequency of chromosomal abnormalities was 85 (23%) which is comparable to other studies (12, 13) (Table 2). Several surveys have been conducted in Iran to determine the frequency of chromosome abnormalities (14, 15). However, our study, for the first time, recruits a relatively large sample size in comparison to previous studies.

Table 2. Comparison of various chromosomal abnormalities found in patients with amenorrhea in different studies with present study.

Reference	Total cases	X chromosomal Aneuploidy (%)	Structural Abnormalities (%)	Male Karyotype (%)	Abnormal Chromosome (%)
(16)	60	60	-	3.3	63.3
(17)	237	12.6	2.9	8.4	24.5
(13)	865	45.5	23.3	31.2	23.4
(18)	10	20	50	30	58.9
(19)	269	82.2	8.17	5.2	54.6
(15)	136	15.6	3.3	5.5	24.4
(6)	600	8.7	9.2	3	21
(20)	108	2.5	20	-	22.5
(7)	108	28.7	3.7	2.77	35.2
(14)	200	14.5	7.5	7	29

In almost all the studies reviewed, the most frequent chromosomal anomaly in amenorrhea patients is abnormalities of X chromosome followed by a male karyotype (6). In our study, the most frequent X-chromosome abnormalities were monosomy (45,X) (34.4%),mosaic (45,X/46XX) and other structural abnormalities of chromosome X (26.6%).

Turner syndrome (pure or mosaic monosomy X) is described to be the prime cause of Amenorrhea and our results were similar to the study of Rajangam (13), 50.8% of the cases of our study proved to be Turner syndromes. Nondisjunction and anaphase lag that take place during meiosis or mitosis are thought to be responsible for most cases to become pure or mosaic Turner syndrome. It also observed that the amenorrhea cases with monosomy of X-chromosome were also short stature. They have also some hormonal changes and absence of secondary sex characters. It is obvious that sex chromosome plays a role in the initiation and maintenance of normal menstruation. The integrity of a critical region in the X chromosome (q13q26) is vital for normal ovarian function (4). This can explain the cause of lack of menstruation in 3 patients with deletion of Xq.

The isochromosome is a mirror-image abnormal chromosome that consists of two copies of either the long arm or the short arm. This abnormal chromosome can cause partial monosomy of the genes on Xp and partial trisomy of genes on Xq. women with 46,X,i (Xq) karyotype have been observed to show streak gonads Complete ovarian failure and partial ovarian failure have been described in 91% and 9%of cases with i (Xq). Moreover, short stature and turner syndrome stigmata are reported to be common with almost complete lack of gonadal development in 46,X,i (Xq) women (21,22).

In 23 cases (27.2 %), male chromosome constitution 46,XY karyotype were detected. Many genetic factors can lead to abnormal women fetal development including pure gonadal dysgenesis with 46,XY, such as X-linked recessive syndrome, autosomal chromosomal anomaly, *SRY*, *DAX1*, *WT-1*, *SOX9*, *SF-1* and *NR5A1* gene mutations (23, 24). It has been revealed that the incidences

of gonadoblastomas, dysgerminomas, and yolk sac tumors vary from 30% to 75% for all women with pure gonadal dysgenesis and 7% to 10% for Turner Syndromes (25, 26). Due to the high risk of neoplastic transformation, it has generally been advised that dysgenetic gonads should be surgically removed as soon as diagnosed.

Autosomal chromosomal abnormalities are rare in patients with amenorrhea. In our study, cases carrying rearrangements between autosomal and/or sex chromosomes were detected in 10 patients (11.8%). We detected 2 cases of the translocations [46,XX,t (13;17) (p21;q25)and 46,XX,t (X; 1) (q13;q24)] in PA. Autosomal translocations have been also reported by several studies representing the possibility of the contribution of autosomal genes in the normal gonadal development (6).

In conclusion, the overall percentage of chromosomal abnormalities indicates that the chromosomal analysis is very essential in all cases of amenorrhea particularly with features of Turner's like and short stature at an early stage. Early diagnosis can help to better manage the disease. Genetic counseling should include the possibility of premature menopause for Turner syndrome patients 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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Conflict of interest

The authors have no conflicts of interest to declare.

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