Absence of Menstruation (Amenorrhea) Due to Chromosomal Abnormalities

Wafaa Abdulzahra Kadhim
Department of Medical analysis-college of health and medical techniques

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ABSTRACT

Absence of menstruation (amenorrhea) is not a disease but symptom that may result from several causes. Cytogenetic investigation have shown the importance of chromosomal abnormalities as a cause of amenorrhea. The present study aimed at performing chromosomal analysis in patients with primary amenorrhea. Cytogenetic investigations were carried out on 55 women with primary amenorrhea by using GTG-band analysis of metaphases from peripheral blood leukocytes. The karyotypes results revealed 74.5%
(n=41) with normal chromosome composition and 25.5% (n=14) showed chromosomal abnormalities. These abnormalities can be classified into three types: x chromosome abnormality (including monosomy X, pure X numerical abnormality, and Turner mosaicism) was observed in 57.14% (n=8) of all chromosomal abnormalities, the other type of abnormal karyotype in this study is a karyotype showed pure XY female found in only 4 cases (28.5%), and the last type of abnormalities which represented in structural abnormalities of X chromosome in 2 cases (14.2%) one of theme showed marker chromosome and the other case showed deletion of X chromosome.

INTRODUCTION

Primary amenorrhea is defined as the absence of menstruation and sexual characteristics in phenotypic women aged 14 years or older or aged 16 years or older if secondary sexual characteristics are absent [1]. Patients with secondary amenorrhea have at least one spontaneous bleeding episode, followed by no menstruation for a minimum of 12 months at or before the age of 42 years old [2]. 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 [3]. Hormonal disorders are the main causes of primary and secondary amenorrhea, common hormonal causes of primary amenorrhea includes chronic systemic disease, hypothalamic-pituitary dysfunction and absent ovarian function [4]. Secondary amenorrhea can be due to pregnancy, hypothalamic-pituitary disorders, polycystic ovarian disease, resistant ovarian syndrome, and absent or premature ovarian failure [2]. Cytogenetic investigation have shown the importance of chromosomal aberration as an important cause of amenorrhea. Different surveys have implicated chromosomal aberration in causing this symptoms in 16-50% of causes [4]. Also genetic or chromosomal causes are the most important as their presence affects subsequent management, for example, girls with XY gonadal dysgenesis have a high (30%) risk of gonadal malignancy and their testes should be removed as soon as possible [2]. For this reason this study is undertaken to determine the frequency and types of chromosomal abnormalities that result in primary amenorrhea.
MATERIAL AND METHODS

All cases were obtained from Baghdad Government and Private Hospitals. Data on family history, clinical features, and laboratory tests were recorded.

A total of 55 women who failed to menstruate before 18 years of age were studied between 2008-2010, their age ranged between 18-36 years, about 1 ml of peripheral blood was collected from each patient in a heparinized container and are used for cytogenetic analysis. About 6-7 drops of blood from each patient was inoculated in 8 ml of RPMI medium supplemented with 2 ml of human plasma, 0.3-0.5 ml of PHA (phytohemagglutinin) and incubated at 37°C with frequent shaking every 24 hrs. After 71 hours of incubation colcemid (0.1 ml) was added with mild shaking and incubated for another (1) hour, the cells were then harvested by hypotonic treatment (20 minutes with 0.075 M KCl at 37°C), fixed and washed thrice with Carnoy’s fixative (methanol and glacial acetic acid in a ratio of 3:1) and casted on wet, pre chilled, grease-free slides. Multiple slides were casted for each sample, slides then stained with freshly made Giemsa stain (1 part Giemsa to 4 parts of Sorensen’s buffer) for 2-3 minutes, slides then exposed to trypsin solution for banding process for 7-10 seconds at room temperature. Stained with Giemsa, air dried. For each sample, 10 metaphase were analyzed and karyotype was interpreted, whenever abnormal karyotype was obtained, a more number of metaphase (25) were analyzed.

RESULTS AND DISCUSSION

About 25.5% of the 55 cases with primary amenorrhea showed an abnormal karyotype. The type and frequency and aberrations involving the sex chromosomes are detailed in table (1). A large number of studies undertaken to ascertain in the frequency of sex chromosomal anomalies in patients presents with primary amenorrhea have shown a wide variants in the incidence of chromosomal anomalies [4,6,7,8,9,10,11,12,13]. Previous estimates of the frequency of chromosomal abnormalities vary from 15.9% [7] to 63.3% [2]. The variation between the present and that of the previous studies may be due to the difference in the selection of patients include for analysis.
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**Table -1 : Constitution Karyotype of Patients with Primary Amenorrhea**

<table>
<thead>
<tr>
<th>Chromosomal abnormalities</th>
<th>Karyotype</th>
<th>No of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Normal karyotype</td>
<td>46,XX</td>
<td>41 (74.55%)</td>
</tr>
<tr>
<td>2 Numerical abnormalities</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a- Monosomy X (pure turner)</td>
<td>45,X</td>
<td>4 (7.27%)</td>
</tr>
<tr>
<td>b- Pure X numerical abnormality</td>
<td>47,XXX</td>
<td>1 (1.81%)</td>
</tr>
<tr>
<td>c- Turner mosaicism</td>
<td>45,X/46,XX</td>
<td>3 (5.45%)</td>
</tr>
<tr>
<td>d- Pure XY female</td>
<td>46,XY</td>
<td>4 (7.27%)</td>
</tr>
<tr>
<td>3 Structural abnormalities</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a- Marker chromosomes</td>
<td>46,X,+m</td>
<td></td>
</tr>
<tr>
<td>b- Deletion of X chromosome</td>
<td>46,X,del(xq)</td>
<td>1 (1.81%)</td>
</tr>
</tbody>
</table>

**Fig. -1: The percentage of normal and abnormal karyotype in 55 patients with primary Amenorrhea.**

- Normal karyotype
- Abnormal karyotype
In patients with abnormal chromosome constituents, 85.7% (n=12) exhibit numerical aberration and 14.3% (n=2) with structural aberration. Fig (2).

Fig (3) shows the distribution of numerical and structural aberration among the patients with abnormal chromosomal constituents. Among the different type of chromosomal abnormalities 28.5% (n=4) of them showed complete Monosomy of (X) chromosome (45, X). The Monosomy of X chromosome is the typical karyotype of Turner’s syndrome [14]. The clinical spectrum of patients with abnormalities of the X chromosome is wide. In
general patients with Monosomy X showed typical Turner’s stigmata while those with structural anomalies showed one or more Turner’s-like features [15]. The differences in clinical manifestation are postulated that genes whose absences determine the somatic features of Turner’s syndrome are distributed along all of Xp and the middle of Xq [16].

The obtained results are in good correlation with that of previous studies as Turner’s syndrome is reported to be the leading cause of primary amenorrhea, also the obtained results further strengthened the role of gene composition on the X chromosome in the normal female physiology and reproduction, for this reason this obtained results are in agreement with studies that conducted in the past. The second frequently occurring karyotype in this study is the mosaic Turner 45, X \(46,XX\) (21.7%) (n=3).

Patients with 45,X \(46,XX\) karyotype also manifest a wide phenotypical variability of them have shown a features of Turner’s syndrome, variable gonad morphology ranging from a testis to a streak gonad [17]. Also it should be informed that women with sex chromosome anomalies, especially having X mosaicism, pregnancy cannot be ruled out [18]. Other X chromosome abnormalities in this study which pure X numerical abnormality, 47, XXX. This abnormality reported in only one case (1.81%). It has been suggested that girls with karyotype 47, XXX have a higher incidence of ovarian failure [19]. Also the extra X can slow down embryonic cell development in a special way [20].

Male karyotype 46, XY present in a significant percentage 28.5%, (n=4) of patient with abnormal karyotype.

The obtained results are in agreement with the previous studies which have reported 25% of cases with Y chromosome constitution [21]. Studies have demonstrated that a female karyotype can occur in XY embryo when testes determining factor (TDF) or other genes in the testes determining pathway are lost, mutated, or compromised [22]. Generally the differential diagnosis of XY females can be classified into four types, based on clinical features, hormonal profile and histology of the gonads. The two commonest and best known are testicular feminizing syndrome (androgen insensitivity syndrome) and pure XY gonadal dysgenesis (Swyers syndrome) [23]. Pedigree studies have shown that testicular feminizing syndrome is inherited through a gene whose expression limited to the male sex [24].
The outstanding feature of this syndrome is feminizing of the patient, absence of fallopian tubes, uterus and cervix [23, 24]. On the other hand, pure XY gonadal dysgenesis is a genetically heterogeneous condition. It appears to be inherited either as a sex-linked or an autosomal- linked gene [24]. The presence of cervix or mullerian system, sexual infantilism and low-normal testosterone levels in these patients serve to differentiate XY gonadal dysgenesis from testicular feminizing syndrome [4]. The pathological significance of these two forms is that they at risk of developing malignant tumors of the gonads [14]. Early diagnosis of XY gonadal dysgenesis and testicular feminizing syndrome and removal of them as soon as possible before malignant growth develops [3].the different structural abnormalities of the X chromosome included deletion of the long arm 46,Xdel(Xq) reported in only one case (7.1%),and another case showed the presence of marker chromosome 46,X+m (7.1%).

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. [25].

On the other hand a gene essential for gonadal function are located on the proximal part of XP, the long arm of X proximal to (Xq13)and/or the long arm of X distal to Xq 26 [20,26]. Large deletion of X q with break points at proximal to q13 are expected to produce gonadal dysgenesis with primary amenorrhea, half of the patients with such deletion have Turner’s syndrome [27]. Also it has been reported that primary amenorrhea patients with 46, X+ mar karyotype may have usually severe phenotypes ,with mental retardation or abnormal facial features [28].

In conclusion this study confirms that chromosomal abnormalities are significant etiological factors in gonadal dysgenesis resulting in primary amenorrhea. The application of molecular cytogenetic and molecular techniques would aid in the delineation of the genetic etiology in cases of amenorrhea presenting with a normal karyotype.

REFERENCES


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