Contribution of chromosomal aberrations to the pathogenesis of primary and secondary amenorrhea: A study from Western Iran

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Objective: Amenorrhea is an abnormal condition characterized by the absence of menstruation in women of reproductive age. According to the World Health Organization, amenorrhea ranks as the sixth leading cause of female infertility. Approximately 2% to 5% of women of reproductive age experience amenorrhea, which can be classified as primary amenorrhea (PA) or secondary amenorrhea (SA). Several studies have named chromosomal abnormalities among the main causes of amenorrhea, though the prevalence of these abnormalities may differ across populations. The objective of this study was to ascertain the frequency and types of chromosomal abnormalities in women with amenorrhea in Kermanshah Province, Iran.

Methods: This retrospective study included patients with PA and SA who underwent standard cytogenetic analysis. We also conducted a review of the literature on chromosomal abnormalities and their prevalence in SA.

Results: Among the 137 cases of PA in this study, 22% exhibited chromosomal abnormalities. Numerical changes were the most common finding (46.6%) in this group, including 45,X, mosaic, and 47,XXX karyotypes. These were followed by the 46,XY karyotype (40%). Of the 51 cases of SA that received chromosomal analysis, abnormalities were identified in only one case. Additionally, our review of the literature revealed that chromosomal aberrations are responsible for 7% of SA cases globally.

Conclusion: In this study, we successfully characterized the cytogenetic causes of PA and SA in a substantial population from Kermanshah Province, Iran.

Keywords: Amenorrhea; Chromosome aberrations; Iran

Introduction

Amenorrhea refers to the absence of menstruation in women of reproductive age. This condition has two types: primary amenorrhea (PA) and secondary amenorrhea (SA). PA is defined as the lack of menstruation by the age of 15 years in conjunction with secondary sexual characteristics and normal growth, or by the age of 13 years in the absence of secondary sexual characteristics. In contrast, SA refers to the absence of three or more consecutive menstrual cycles in individuals who previously had regular periods, or the absence of menstrual cycles for more than 6 months in women with a history of irregular periods [1]. A variety of factors can cause amenorrhea, and these are common to both PA and SA. However, the relative contribution of each underlying cause varies between these types [2,3].

From a genetic perspective, amenorrhea can be attributed to single-gene and chromosomal defects. Mutations in genes such as the gonadotropin-releasing hormone (GnRH) receptor (GNRHR), kisspeptin (KISS1) and its receptor (KISS1R), and neurokinin B (TAC3) and its receptor (TACR3)—all of which regulate the release of GnRH—can
lead to amenorrhea. Furthermore, over 25 genes have been associated with congenital hypogonadotropic hypogonadism (CHH) and Kallmann syndrome. These genes account for approximately 50% of CHH cases and include anosmin 1 (ANOS1; located at Xp22.32), fibroblast growth factor Receptor 1 (FGFR1; 8p11.23-p11.22), prokineticin receptor 2 (PROKR2; 20p12.3), and prokineticin 2 (PROK2; 3p13) [4,5].

Chromosomal abnormalities are another common cause of amenorrhea [6]. These abnormalities may be either numerical or structural. Various studies have estimated that chromosomal abnormalities occur in 10% to 25% of PA cases. For instance, Turner syndrome (45,X) is one of the most common chromosomal aberrations leading to primary ovarian failure. The incidence of Turner syndrome is about five in 100,000 women [7].

Early referral for cytogenetic testing is strongly recommended to identify underlying chromosomal abnormalities in patients with amenorrhea. This step is crucial for effective disease management, as certain abnormalities, such as the presence of a Y chromosome, can increase the risk of gonadal malignancy and necessitate additional interventions. Given the key role of chromosomal abnormalities in the development of amenorrhea and the subsequent counseling and management of patients, accurately determining the frequency of these abnormalities is paramount. Studies have reported varying rates, ranging from 16% to 64% [8]. These discrepancies highlight the need to understand the frequency and distribution of chromosomal abnormalities among individuals affected by amenorrhea in different populations. In this retrospective study, we aimed to gather detailed information on the frequency of chromosomal abnormalities in PA and SA through a comprehensive analysis of a relatively large population in Kermanshah Province, Western Iran.

Methods

This descriptive study included all patients diagnosed with amenorrhea by an expert gynecologist who were referred to the cytogenetics department of the reference laboratory in Kermanshah for chromosomal testing between 2008 and 2023. We excluded pregnant and lactating women, as well as those whose amenorrhea developed following recent ovarian surgery, radiation, or medication. Cases of polycystic ovarian syndrome and hyperprolactinemia were also omitted, along with patients who declined to participate in the study.

Participants were divided into PA and SA subgroups in accordance with published guidelines [9]. All participants provided their informed consent. The study received approval from the Ethics Committee of the School of Medicine at Kermanshah University of Medical Sciences (IR.KUMS.MED.REC.1401.115).

For all cases, metaphase chromosome preparation and G-banding were conducted in accordance with the standard protocol [10]. A minimum of 50 cells were analyzed in each case, and the karyotype results were reported in line with the 2016 recommendations of the International System for Human Cytogenetic Nomenclature [11].

To obtain an overall estimate of the frequency of chromosomal aberrations in SA, we conducted a comprehensive review of the medical literature. We utilized the PubMed and Scopus databases to identify articles published up to July 30, 2023. Our search terms included “secondary amenorrhea” [all fields] AND “chromosome abnormality” [all fields]. We excluded non-English results and papers for which the full text was not available.

Results

In total, 189 cases of amenorrhea were referred to the cytogenetic department of the reference laboratory for chromosomal examination. This cohort comprised 138 cases of PA and 51 cases of SA. The mean age at referral for the PA group was 20.1 years, compared to 28.66 years for the SA group (Table 1).

Of the 137 cases of PA in this study, 78% exhibited a normal karyotype, while 22% presented with chromosomal abnormalities. These abnormalities included numerical aberrations, followed by a 46,XY karyotype and structural aberrations (Table 2). As detailed in Table 2, numerical aberrations were the most common, accounting for 46.6% of abnormalities. These included 45,X, mosaic, and 47,XXX karyotypes. The second most frequent finding in the PA group was the

Table 1. Participant demographics

<table>
<thead>
<tr>
<th>Type of amenorrhea</th>
<th>Number</th>
<th>Age (yr) (average ± SD)</th>
<th>Age (yr) (range)</th>
<th>p-value (min–max)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary</td>
<td>138</td>
<td>20.1 ± 5.42</td>
<td>13–39</td>
<td>&lt;0.0001 (6.57–10.54)</td>
</tr>
<tr>
<td>Secondary</td>
<td>51</td>
<td>28.66 ± 7.764</td>
<td>15–44</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>189</td>
<td>22.4</td>
<td>13–44</td>
<td></td>
</tr>
</tbody>
</table>

SD, standard deviation.

Table 2. Frequency of chromosomal aberrations among primary amenorrhea subgroup

<table>
<thead>
<tr>
<th>Karyotype</th>
<th>Number</th>
<th>Frequency in all cases (%)</th>
<th>Frequency in aberrations (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal karyotype</td>
<td>107</td>
<td>78</td>
<td>-</td>
</tr>
<tr>
<td>Chromosomal aberrations</td>
<td>30</td>
<td>22</td>
<td>-</td>
</tr>
<tr>
<td>Sex reversal (46,XY)</td>
<td>12</td>
<td>8.8</td>
<td>40</td>
</tr>
<tr>
<td>Numerical aberrations 45,X</td>
<td>14</td>
<td>10.2</td>
<td>46.6</td>
</tr>
<tr>
<td>45,X</td>
<td>6</td>
<td>4</td>
<td>20</td>
</tr>
<tr>
<td>Mosaic karyotypes</td>
<td>4</td>
<td>3.6</td>
<td>13.3</td>
</tr>
<tr>
<td>47,XXX</td>
<td>4</td>
<td>2.8</td>
<td>13.3</td>
</tr>
<tr>
<td>Structural aberration</td>
<td>4</td>
<td>2.8</td>
<td>13.3</td>
</tr>
</tbody>
</table>
46,XY karyotype (40%), which is associated with the sex reversal phenotype. Additionally, four cases of PA were found to have chromosomal structural aberrations: 46,X,i(X)(q10), 46,Y,i(X)(q10), 46,X,del(X)(q21), and 46,XX,t(7;12)(p14;q12). A partial karyotype illustrating chromosome X aberrations is provided in Figure 1.

Normal chromosomal variants were detected in 21 cases (15%), primarily consisting of an increase in the length of the pericentric heterochromatin (qh+) in 18 cases and heteromorphisms of the acrocentric chromosomes in three cases.

Of the 51 cases of SA subjected to chromosomal analysis, chromosomal abnormalities were identified in only one case. This patient was a 35-year-old woman with a history of regular menstrual cycles who presented with amenorrhea that began at the age of 30. In addition to SA, she exhibited short stature, hypothyroidism, and mild intellectual disability. Chromosomal analysis revealed a karyotype of 46,XX,del(X)(p11). The remaining cases of SA did not exhibit any pathogenic chromosomal alterations. However, non-pathogenic variants were detected in 11% of the cases, mainly involving a lengthening of the pericentric heterochromatin (qh+).

Regarding the overall contribution of chromosomal aberrations to the pathogenesis of SA, we included a total of 1,416 SA cases in our literature review. As indicated in Table 3, chromosomal aberrations represented the disease etiology in 7% of SA cases. Numerical aberrations were the most common, comprising 58% of cases, followed by structural aberrations at 34%. Notably, a substantial proportion of these aberrations were mosaic karyotypes.

Discussion

While the etiology of amenorrhea is highly heterogeneous, genetic factors, including chromosomal and single-gene abnormalities, account for approximately 40% of cases [8]. Our investigation focused on the prevalence of chromosomal abnormalities among women with PA and SA in Kermanshah Province.

In the present study, 78% of patients with PA exhibited a normal karyotype. This finding compares with a similar study conducted in northeastern Iran involving 180 PA cases, with 75.55% displaying a normal karyotype [12]. Another study of an Iranian population reported a normal karyotype rate of 77.6% [13]. In southwestern Iran, the frequency of a normal karyotype among patients with amenorrhea was found to be 80% [14]. Studies of non-Iranian populations have reported varying frequencies of normal karyotype in patients with PA, with figures ranging from 58.29% in Mexico [15] to 89% in India [16]. These differences may arise in part from variations in selection criteria, sample size, and genetic background.

Regarding SA, only one case was identified as having a chromosomal abnormality, specifically a deletion on the short arm of the X chromosome. A comparable study of an Indian population, which comprised 852 cases of PA and 127 cases of SA, found that 7.09% of the SA cases exhibited an abnormal karyotype. Notably, this Indian study also reported two cases of SA with a deletion on the short arm of the X chromosome (46,X,del(Xp)) [17]. Therefore, it can be inferred that the deletion of Xp is a chromosomal aberration that contributes to SA. Additional karyotypes identified in the Indian SA population included 45,X, 47,XXX, 45,X/46,X,i(Xq), and 45,X/46,X,r(X). In a separate study from India, only one of 22 SA cases presented with an abnormal karyotype, which was 45,X [18]. The prevalence of chromosomal abnormalities among patients with SA in the Iraqi population was found to be 6.6% [19]. Based on our analysis of the available data on SA, the overall rate of chromosomal abnormalities in SA is approximately 7%, with the majority of these cases being mosaic in

Table 3. Overall frequency of chromosomal aberrations among secondary amenorrhea patients found through literature

<table>
<thead>
<tr>
<th>Chromosomal aberration</th>
<th>Number (n = 1,416)</th>
<th>Frequency in all cases (%)</th>
<th>Frequency in aberrations (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex reversal (46,XY)</td>
<td>7</td>
<td>0.5</td>
<td>7</td>
</tr>
<tr>
<td>Numerical aberrations (n = 58)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-mosaic</td>
<td>13</td>
<td>0.9</td>
<td>13</td>
</tr>
<tr>
<td>Mosaic</td>
<td>45</td>
<td>3.17</td>
<td>45</td>
</tr>
<tr>
<td>Structural aberration (n = 34)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-mosaic</td>
<td>27</td>
<td>1.9</td>
<td>27</td>
</tr>
<tr>
<td>Mosaic</td>
<td>7</td>
<td>0.4</td>
<td>7</td>
</tr>
<tr>
<td>Total</td>
<td>99</td>
<td>7</td>
<td>100</td>
</tr>
</tbody>
</table>

Figure 1. Partial karyotypes of X chromosome aberrations. (A) Trisomy X. (B) Monosomy X. (C) i(X)(q10). (D) del(X)(q21). (E) XY complement in a phenotypically female patient.
nature (Table 3).

Within the PA population, the most frequently observed chromosomal abnormality was the 46,XY karyotype present in phenotypically female participants (40%), followed by a pure 45,X karyotype (20%). This result is noteworthy given that it was derived from a relatively homogeneous genetic population and contrasts with findings from similar studies, in which the non-mosaic Turner karyotype has typically been the most prevalent chromosomal aberration among patients with PA. For instance, two analogous studies conducted in the northeast and southwest regions of Iran reported the non-mosaic karyotype 45,X as the most common chromosomal abnormality in these patients, with frequencies of 34.4% and 43%, respectively [20,21]. Furthermore, research in non-Iranian populations has also identified the non-mosaic karyotype 45,X as the predominant chromosomal finding in patients with PA [8,15,19,22].

Several genetic factors can cause a 46,XY fetus to develop a female phenotype. These factors include mutations in the sex determining region Y (SRY), nuclear receptor subfamily 0 group B member 1 (NR0B1), Wnt family member 4 (WNT4), SRY-box transcription factor 9 (SOX9), splicing factor 1 (SF1), and nuclear receptor subfamily 5 group A member 1 (NR5A1) genes [23]. As such, 46,XY individuals with a female phenotype can be further screened for these mutated genes. Additionally, the risk of developing gonadal tumors (including gonadoblastomas, dysgerminomas, and yolk sac tumors) in women with complete gonadal dysgenesis is reported to be between 35% and 75% [24]. Given the high risk of neoplastic transformation, the immediate surgical removal of dysgenic gonads is strongly recommended upon diagnosis.

One of the structural aberrations observed in the present study was a reciprocal translocation between chromosomes 7 and 12, noted as 46,XX,t(7;12)(p14;q12). Such autosomal structural aberrations are infrequently observed in patients with amenorrhea. A comparable study from Mashhad reported a reciprocal translocation of 46,XX,t(13;17)(p21;q25). Although reports of translocations between autosomal chromosomes in patients with PA are scarce, they have been documented in the literature [20,25]. The link between autosomal translocations and PA must be approached with caution, as it may simply be incidental. Nonetheless, it is possible that such a translocation may disrupt a gene that is involved in sexual development, resulting in amenorrhea. Further research is necessary to explore this suggestion.

Heteromorphisms are normal chromosomal variations within the population that typically lack clinical implications. In the present study, chromosomal variants were identified in 15% of PA cases and in 11% of SA cases. In 2022, Dey et al. [26] conducted research involving 178 individuals with amenorrhea to explore the relationship between chromosomal heteromorphisms and the incidence of amenorrhea. The findings revealed that 10.11% of the participants exhibited regions with increased lengths of heterochromatin, a proportion that is notably higher than that of the general population studied. Consequently, the researchers suggested that changes in heterochromatin regions could influence key processes such as chromosome architecture, histone modifications, and the regulation of gene expression, potentially leading to ovarian dysfunction and resulting in amenorrhea. Nevertheless, due to the typically benign nature of these variants, additional research is required to clarify their role [26].

The prevalence of the isochromosome mosaic karyotype ranges from 8% to 9% [27], and evidence suggests that patients with X isochromosome mosaicism tend to exhibit a comparatively mild phenotype. Nonetheless, establishing a definitive correlation between karyotype and phenotype in mosaic cases remains a challenge. In the present study, we encountered a case with the following mosaic karyotype: mos45,X[37]/46,X,i(X)(q10) [11]. This individual presented with PA, short stature (146 cm), and a normal body mass index (BMI; 23 kg/m²). Ultrasound examination revealed uterine hypoplasia, a thin and unmeasurable endometrium, and ovaries that were smaller than normal. Physical examination showed no webbed neck, with normal breast development and pubic hair. In contrast, another case with a mosaic karyotype of mos45,X[37]/46,X,i(X)(q10) [13] exhibited a different set of phenotypes. This patient was also short (134 cm), had a normal BMI (20.67 kg/m²), and presented with uterine hypoplasia. However, she also had alopecia, absent ovaries, and Tanner stage 1 pubic hair and breast development [28]. The observed phenotypic variation may stem in part from different distributions of the 45,X and i(X) cell lines across various tissues, since only peripheral blood is analyzed during routine karyotyping. Other genetic factors that may modify the phenotype could also play a role.

In this study, we identified the etiology of amenorrhea in 22% of patients with PA, whereas in SA, only one case (1.9%) exhibited a chromosomal aberration associated with the condition. These findings could pave the way for improved disease management and genetic counseling for patients with amenorrhea. However, our study was limited by incomplete access to the clinical findings of all participants, preventing us from accurately delineating the correlation between phenotype and karyotype. This limitation should be considered, and the incorporation of such an analysis is strongly recommended for future research.

**Conflict of interest**

No potential conflict of interest relevant to this article was reported.
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References

