

Original Article

Chromosomal Abnormality in Patients with Secondary Amenorrhea

Akbar Safai MD¹, Mohammad Vasei MD², Armin Attaranzadeh MD¹, Fariborz Azad DVM¹, Narjes Tabibi MS¹

Abstract

Background: Secondary amenorrhea is a condition in which there is cessation of menses after at least one menstruation. It is a symptom of different diseases, such as hormonal disturbances which range from pituitary to ovarian origin, as well as chromosomal abnormalities. Knowledge of the distinct cause of secondary amenorrhea is of tremendous benefit for the management and monitoring of patients. In this study, we determine the chromosomal abnormalities in patients with secondary amenorrhea in Southwest Iran.

Methods: We selected 94 patients with secondary amenorrhea who referred to our Cytogenetic Ward from 2004 until 2009. For karyotyping, peripheral blood lymphocyte cultures were set up by conventional technique.

Results: In this study, 5.3% (n=5) of patients with secondary amenorrhea presented with chromosomal abnormalities, of which all contained an X element. The chromosomal abnormalities were:

i) 45, X (n=1); ii) 47, XXX (n=1); iii) 45, X [13]/ 45, Xi(X)q[17] (n=1); iv) 45, X[12]/46,X,+mar[12] (n=1); and v) 46,X,del(Xq)(q23q28) (n=1).

Conclusion: Our study revealed that some causes of secondary amenorrhea could be due to chromosomal abnormalities. Therefore, cytogenetic studies should be important tests in the evaluation of patients with secondary amenorrhea.

Keywords: Chromosomal abnormality, cytogenetic study, karyotyping, secondary amenorrhea

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Introduction

Amenorrhea is classified as either primary or secondary. Primary amenorrhea is defined as the lack of onset of menstruation by age 14–16 according to developmental status.^{1,2} Secondary amenorrhea is a condition demonstrated by the absence of menstruation for six months or 3 previous cycle intervals in a female who has had at least one previous menstruation. Among the general population, amenorrhea is detected in 2%–5% of all females of childbearing age.¹ There are many causes for secondary amenorrhea, of which hormonal disorders are the main cause.² Hormonal disorders include hypothalamic-pituitary disturbances, polycystic ovary, resistant ovary syndrome, and premature ovarian failure. Understanding the distinct cause of secondary amenorrhea is of tremendous assistance in management and monitoring these patients. Genetic or chromosomal abnormalities are one of the most important causes of secondary amenorrhea, and may be either a single gene disorder, chromosomal abnormality or multifactorial.^{1,2} Chromosomal abnormalities are divided into numerical, structural, and mosaicism.¹ In some studies from various parts of the world, numerical or structural chromosomal abnormalities have been reported at levels ranging from 3.8% to 44%.^{1–11} Such a wide variation is likely due to varied selection criteria of patients in different studies.

In this study, we evaluated cytogenetic findings in patients diagnosed with secondary amenorrhea who resided in Southwest Iran.

Materials and Methods

In a cross-sectional study, all women with secondary amenorrhea who referred to the Cytogenetic Ward of the Department of Pathology, Shiraz University of Medical Science, Shiraz, Iran from 2004 to 2009 were selected. Secondary amenorrhea was defined as the absence of menstruation after at least one menstruation that occurred prior to their complaint.² Clinical and laboratory data were obtained from hospital records or the referring physician. Family history was reviewed during genetic counseling and family members were tracked back three generations. Secondary sexual characteristics and hormonal conditions were recorded. Serum luteinizing hormone (LH) and follicle stimulating hormone (FSH) concentration data were retrospectively analyzed from hospital records. If serum LH and FSH levels were checked several times, the maximal value was used in the analysis. Blood samples were obtained with heparin syringes and lymphocytes were cultured in RPMI 1640 basal media and 10% fetal calf serum (Gibco, Invitrogen, USA) and treated with 0.1 µg/ml colcemide (Gibco, Invitrogen, USA) after a 72 hour incubation period. Subsequently, metaphase chromosomes were spread and stained using standard G-banding techniques.

In every patient, at least 15 metaphase chromosomal complexes were examined and if mosaicism was suspected, further metaphases were examined.

Results

A total of 94 women with secondary amenorrhea referred to this center for karyotype analysis from other genetic clinics, gynecological practitioners, and other services. Patients' ages ranged from 16 to 41 years with a mean of 26.5 years. They had men-

Authors' affiliations: ¹ Pathology Ward, Shiraz Medical University, Shiraz, Iran, ²Teheran Medical University, Tehran, Iran.

Corresponding author and reprints: Armin Attaranzadeh MD, Pathology Ward, Shiraz Medical University, Setad sq., Shiraz. Tel: +98-511-228-8813, Fax: +98-511-228-8812, E-mail: armin.atta@gmail.com.

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stuation one time prior to their diagnosis and the delay in menstruation ranged from 6 months to 7 years.

Normal karyotype was present in 89 women (94.7%); 5 cases (5.3%) had at least one chromosomal abnormality, as shown below:

- i) 45, X (n=1); ii) 47, XXX (n=1); iii) 45, X [13]/ 45, Xi(X)q[17] (n=1); iv) 45, X[12]/46,X,+mar[12] (n=1); and v) 46,X,del(Xq)(q23q28) (n=1).

A total of 47 individuals (50%) had high levels of LH (23–90 mIU/mL; mean: 63 mIU/mL) and FSH (28–134 mIU/mL; mean: 74 mIU/mL). In patients with chromosomal abnormalities, there were abnormal levels of LH (mean: 72 mIU/mL) and FSH (mean: 88 mIU/mL) in 4 cases (80%).

In 33 women, there was a history of hormone therapy, from which 29 responded to treatment and 4 were unresponsive. No family history was present in the patients. None of patients with chromosomal abnormalities had histories of hormone therapy.

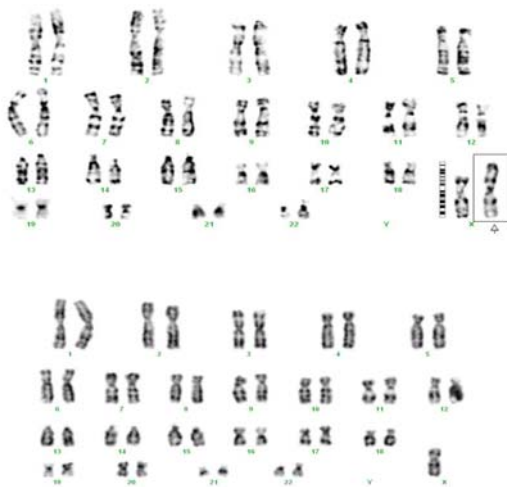


Figure 1. Chromosomal abnormality in one patient with 45, X[13]/45,Xi(X)q[17] karyotype.

Discussion

Secondary amenorrhea is one of the important reasons for patient referral to an endocrine or gynecologic clinic. Karyotype examination is a useful tool in cytogenetic laboratories. In secondary amenorrhea, a history of pregnancy does not exclude cytogenetic abnormalities and after exclusion of non-genetic cases, patients should receive genetic counseling.² Secondary amenorrhea usu-

ally is caused by cortical or hypothalamic disorders with an endocrine abnormality or ovarian dysfunction.

Chromosomal abnormality is a rare, but important cause of secondary amenorrhea that can be detected by cytogenetic analysis. The prevalence of chromosomal abnormalities in secondary amenorrhea varies from 3.8% to 44% in different parts of the world.

Wong et al.² evaluated 312 patients and compared their results with other studies (Table 1). According to this study, the prevalence of chromosomal abnormalities was 9.9%, whereas in other studies it varied from 3.8% to 44%. There is no specific cause for this wide variation of results among different ethnic groups, but may be due to different selection criteria as well as different population genetics throughout the world. One selection criteria is the genetic study in patients with increased gonadotrophin levels.⁴

In our study, the frequency of chromosomal abnormalities was 5.3% which is comparable to other studies (Table 1). Chromosomal abnormality may be numerical or structural. In a previous study, numerical anomaly and their mosaicism have been shown to be the most common causes of chromosomal abnormalities, with a numerical to structural ratio of 22:9.² In our study, 2 cases (40%) had numerical anomalies 47,XXX and 45,X; 2 (40%) had mosaicism [45,X/46X,+mar and 45,X/46,Xi(Xq)]; and 1 case (10%) had a structural anomaly 46,X,del(Xq)(q23 q28).

Premature ovarian failure may be secondary to X chromosome deletion or translocation. POF1 and POF2 genes located at Xq, are the genes essential for normal ovarian function.^{2,12} In our results, all patients had X chromosome abnormalities, of which 4 (80%) had total or partial deletions of the X chromosome.

In secondary amenorrhea, the frequencies of chromosomal abnormalities were 45,X (40%–50%); X mosaicism (25%–36%); X structural (8%); and 46,XY female (16%).^{1,11}

Autosomal abnormalities are rare. van Niekerk showed one case of X/autosome translocation⁵ and Jyothy reported one case of 46,XY,13p+ in secondary amenorrhea patients.³ In this study, we did not find any similar abnormalities.

A comparison of primary and secondary amenorrhea in our region showed a higher prevalence of chromosomal abnormalities in primary amenorrhea (20%) than in secondary amenorrhea (5.3%).¹³ Our results for secondary amenorrhea cytogenetic abnormalities were much less than other studies of primary amenorrhea, in which the frequency ranged between 24.5–27.3.^{5,8}

Currently, chromosome analysis is not limited to research, but is increasingly studied in many clinical disorders. Many indications exist for karyotyping, of which one is amenorrhea (primary or secondary). In secondary amenorrhea, patients with high gonado-

Table 1. Number and frequencies of secondary amenorrhea among different ethnic populations.

Population	US	Chile	Germany	Iran	Turkey	Shanghai	India	Hong Kong	South Africa
Cases	30	47	15	94	9	18	339	312	103
46,XX	26	32	10	89	8	10	324	281	99
Abnormal karyotype	4	15	5	5	1	8	15	31	4
	(13%)	(32%)	(33%)	(5.3%)	(11%)	(44%)	(4.4%)	(9.9%)	(3.8%)
45,X	—	5	—	1	—	—	—	5	—
Mosaic 45,X	—	—	—	2	—	—	—	11	—
46,Xi	—	—	—	—	—	—	—	1	—
47,XXX	—	—	—	1	—	—	—	3	—
46,X/delX	—	—	—	1	—	—	—	6	—
Others	—	—	—	—	—	—	—	5	—
	Devi and Benn ⁷	Castilo et al. ¹⁰	Opitz et al. ¹¹	This study	Temocin et al. ⁸	Lin and Yu ⁹	Jyothy ³	Wong ²	van Nierkerk ⁵

tropin levels that are idiopathic who present with clinical signs of Turner Syndrome are advised to undergo karyotyping for evaluation and monitoring. Karyotype studies in secondary amenorrhea show a significant prevalence of chromosomal anomalies, thus early cytogenetic investigation is necessary to guide further treatment. Our observations have revealed the necessity for cytogenetic examination in all women of reproductive age who present with symptoms of secondary amenorrhea. We also recommend fluorescent in situ hybridization (FISH) for patients with secondary amenorrhea because in routine cytogenetic studies, low levels of mosaicism cannot be ruled out.

To better evaluate and determine cytogenetic abnormalities in both primary and secondary amenorrhea, a larger geographical study in Iran is recommended.

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