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CYTOGENETIC ANALYSIS IN WOMEN WITH PRIMARY AND SCONDARY AMENORRHEA IN IRAN: RETROSPECTIVE STUDY ON 110 PATIENTS

H. Afshar[1], R. Najafipour[2], J. Ansari[2], N. Karimi[3], M. Jalilvand[2,*]

[1]Qazvin University of Medical Sciences, Qazvin, Iran

[2] Cellular and Molecular Research Center, Qazvin University of Medical Sciences, Qazvin, Iran

[3]Department of Molecular Biology, Pasture Institute of Iran, Tehran, Iran

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ABSTRACT

The absence of menstrual periods for one or more periods is called amenorrhea. Primary amenorrhea means the lack of its occurrence until the age of 16 years. Secondary amenorrhea refers to situations that a person has experienced menstruation periods in the past and then stops its occurrence. Amenorrhea is a symptom, not a disease that can cause due to disorders of hypothalamus and pituitary glands, disorders of sexual glands and deformities of the uterus and ovaries. Due to the clinical importance of reproductive in life and non-reproductive of women amenorrhea due to ovarian function, this study has been conducted in order to determine the prevalence and type of chromosomal abnormalities in women referred to the Cytogenetic Laboratory of Cellular and Molecular Research Center, in University of Medical Sciences of Qazvin. Karyotype analysis and its relationship with phenotype were performed on 110 Iranian female patients with primary and secondary amenorrhea. Metaphase chromosomes were prepared and analyzed by G-banding technique and were evaluated to examine the mosaicism of 100 cells of lymphocytes cells.

Author Correspondence, e-mail: rahamj7@yahoo.com

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Karyotype of 91 women (82.72%) was normal and chromosomal abnormalities in 19 patients out of 110 patients (17.37%) were observed with primary and secondary amenorrhea. Primary amenorrhea was observed in 17 of these patients, two of whom also had a secondary amenorrhea. Monosomy chromosome X (6 Cases- 31.57%) and Monosomy chromosome Y (6 Cases- 31.57%) was the most common disorders observed in the women with primary amenorrhea. Monosomy mosaic and trisomy chromosome X were observed in 2 cases of women with secondary amenorrhea (21.81% of the whole statistical sample). The overall prevalence of chromosomal abnormalities was between 15.9 % and 63.3% among patients with primary amenorrhea, that our findings in chromosomal analysis of these women (19.76%) confirm it.

Keywords: cytogenetic, primary amenorrhea, secondary amenorrhea, chromosomal abnormalities.

1. INTRODUCTION

Primary amenorrhea or absence of menstrual periods occurs when a young girl still has not been started her menstrual periods until age 16 and secondary amenorrhea occurs in women who have menstrual periods and then it has been stopped [1; 2].

Primary amenorrhea less than is involved 1% and secondary amenorrhea is involved around 5 to 7 percent of teenage girls in the United States .It should be noted that there is no evidence that the incidence of amenorrhea varies according to the population and ethnic diversity[3].

The most common reasons of primary amenorrhea are disorders in the hypothalamus and pituitary (27.8%), disorders of glands sexual / ovarian (50.4%), and genital and urinary abnormalities (21.8%).Cytogenetic studies indicate the important role of chromosomal abnormalities in creating of amenorrhea. [4; 5; 8; 6]

So, half the primary amenorrhea cases that are created by sexual glands /ovary disorders often are created by abnormal sex chromosome (X). Usually sexual glands disorders are created by especially the Turner syndrome (TS). But also amenorrhea is seen in sexual glands disorders XX 46 and XY 46 (syndrome Swyer), or intersex disorders (insensitivity syndrome to complete androgen of CAIS) and mixed sexual glands disorders (MGD). [7;3].

In contrast most cases of secondary amenorrhea (SA) are created by chromosomal abnormalities such as monosomy X or trisomy of chromosome XXX. [3]. The aim of this retrospective analysis was to estimate the frequency of occurrence the chromosomal abnormalities in women referred to the Cytogenetic Laboratory of Cellular and Molecular Research Center, in University of Medical Sciences of Qazvin. All patients were evaluated clinically and were evaluated with G-banding technique by cytogenetic method.

1.1. Research methodology

Peripheral blood samples were prepared from 110 women with primary and secondary amenorrhea during the period December 2010 to November 2015, among women referred to the Cytogenetic Laboratory of Cellular and Molecular Research Center, in University of Medical Sciences of Qazvin. Inclusion criteria included confirmation of primary amenorrhea or secondary amenorrhea (cessation of menstruation for more than 6 months).

To prepare slides of chromosomes of each person (karyotyping) was used method Rooney and Czepulkowski [8] with changes. 2 ml of peripheral blood of each patient were collected in the heparinized syringes. Of each sample ,10 drop of blood of patients were cultured with 5 cc of 20% culture medium RPMI (GIBCO, UK), 2% solution of serum FBS (GIBCO, UK),1% PHA (GIBCO, UK), penicillin antibiotics mixture / streptomycin (GIBCO, UK) in the sterile media. Then it was incubated for 72 hours in a 37Cº incubator without CO2. Cell proliferation with the addition of 1% volume of Kalsmyd (GIBCO, UK) was stopped after 72-hour culture. During this period, cell culture was incubated for 20 minutes at 37Cº.

Then centrifuge and the supernatant solution from sedimentation of cells were removed and 10 cc of 37Cº KCL hypotonic solution for lysis of red blood cells added to it and was incubated for 20 minutes at 37 Cº. Then the cells were fixed and washed by fixative solution of acetic acid-methanol (1: 3) (Merck, Germany) at laboratory temperature. Stabilization of slides was performed for 3 times, each time after sediment of lymphocytes by centrifugation with a round of rpm of 1200 for 10 minutes. At the end the solution containing white blood cells (lymphocytes) must be colorless and clean, otherwise it can be repeated the fixation operation . Finally, fixed cells were dropped on the slides. Slides were prepared to stain after drying. Prepared slides were transported to lose their excess water at 37 Cº for a few days. Then each

slide was placed first for 40-60 seconds at 37 Cº bonding solution (containing the solution 95% Hanks (HBSS), containing 5% pancreatin (Sigma, Germany).

Immediately after washing each slide was placed in the first 37 Cº washing solution (containing 95% normal saline and 5% bovine serum FBS) for 40-60seconds and then at a laboratory temperature in the second washing solution (containing 50 cc normal saline) for 7-8seconds. Then each slide in the special staining box contains 3-5 percent Giemsa (Merck, Germany) was stained for 4-5 minutes. Immediately after staining each slide was washed with distilled water, dried at laboratory temperature and was studied by light microscopy (Cytovision, Applied Imaging). At least 50 metaphases were evaluated for each patient and according to standard protocols have band resolution of 400 to 450. Blood had been only studied tissue routinely.

That is why this study had been limited to blood analyzes. Data analysis was performed by using Fisher test and was considered a significance level of 0.05%.

2. STAINING BOX

2.1. Findings

A number of 110 women entered to study with confirmation the 86 cases of primary amenorrhea (78.2%) and 24 cases of (21.8%) secondary amenorrhea under 40 years old and with FSH> 40mlU / ml. The mean age of the patients was 37.63. 17 women (19.76%) with primary amenorrhea had abnormal karyotype. 6 patients (35.29%) were identified with Monosomy chromosome X.

One patient (5.8%) was observed also with Monosomy X chromosome and Inversion of chromosome 9. Inversion of chromosome 9 and chromosome X in the 2 patients (11.76%) were observed. Isochromosome and remove part of the long arm of chromosome X, each one (5.8%) was observed in one patient. 6 patients were detected also (35.29%) with karyotype, XY 46 with mixed sexual glands disorders (MGD) (Table 1).

Table 1. Chromosomal abnormalities in 17 Patients with primary amenorrhea

Frequency	Diagnosis	Type of Chromosomal abnormalities
		X chromosome homogenous monosomy
35.29%	TS	45,X
5.8%	TS	45,X,inv(9)(p11 q13)
		X chromosome unbalanced structural abnormalities
5.8%	TS	46,X,i(Xq)(q10:q10)
5.8%	TS	46,X,del(Xq)(q13)
		X chromosome balanced structural abnormalities
11.76%	PA	46,XX, inv(9)(p11 q13)
		46,XY sex-reversal syndrome
35.29%	MGD	46,XY

Most abnormalities were observed in the patients with primary amenorrhea of Monosomy of chromosome X, that all of these patients had clinical symptoms of Turner syndrome. Short stature and delayed puberty and lack of incidence secondary sexual characteristics were of important clinical symptoms of these patients. In one of these patients in addition to X Monosomy, was observed the Inversion of chromosome 9 that the primary amenorrhea with delay of puberty was reported of clinical symptoms of this patient. Inversion of chromosome 9 and chromosome X was detected in two other patients with clinical symptoms of primary amenorrhea (Fig 1).

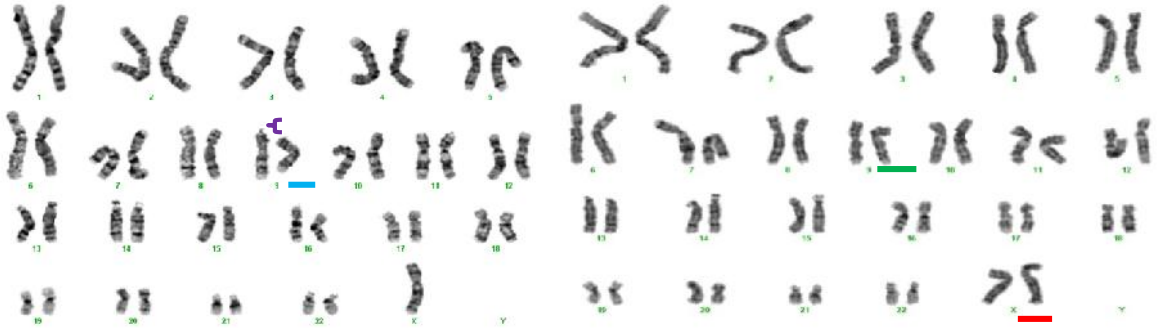

Fig.1. A: Karyotype: 46, XX, inv (9) (p11q13) an apparently normal female karyotype. However one chromosome 9 had a pericentric inversion around centromere. This is usually considered as a normal population variant. / B: Karyotype: 45, X, inv (9) (p11q13) A female karyotype with only one X chromosome which is consistent with Turner syndrome. However, one chromosome 9 had a pericentric inversion around its centrome.

It should also be noted that patients of Monosomy of X chromosome with clinical symptoms of Turner syndrome such as short stature, primary amenorrhea, the absence of secondary sexual characteristics, showed high serum levels of LH and FSH. Two cases of these women with Turner syndrome were developing to hypogonadism in addition to primary amenorrhea. Also, in this study, long arm of chromosome X was detected in the Isochromosome disease and in another disease, was detected the removal of part of the long arm of chromosome X (Fig 2).

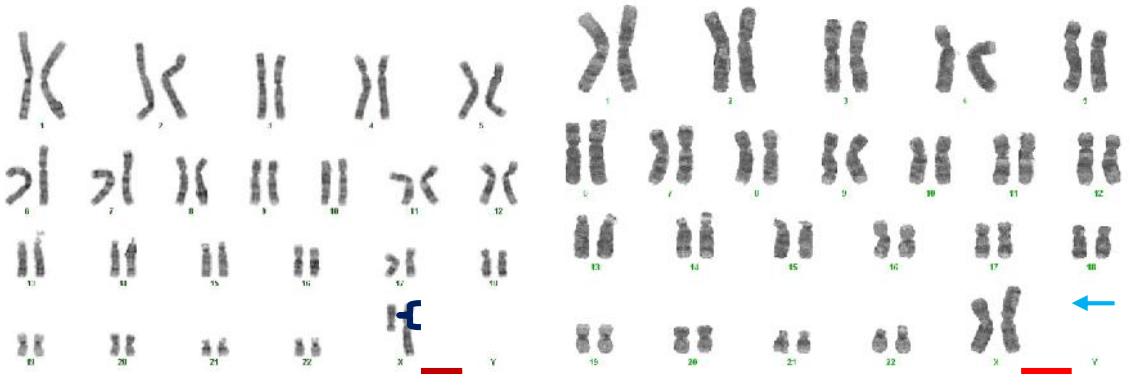

Fig.2. A: Karyotype: 46,X,i(X)(qter→q10::q10→qter) An abnormal female karyotype with an isochromosome for long arm of X chromosome./ B: Karyotype: 46,X,del(X)(q13) An abnormal female karyotype with a deletion in the long arm of one X chromosome.

In addition, karyotypes of 6 persons (35.29%) of women with primary amenorrhea, XY 4 were diagnosed that these women had clinical, variable symptoms and features and sexual glands disorders.

Three cases of these women were sisters of 26 25.36 years old and had clinical symptoms of primary amenorrhea, vaginal blind and testis inguinal, (Figure 3). However, by genealogy analysis of this family, it became clear that there is no family history of developing to this disease in the family of their father and mother. These sisters had also male sexual organ in addition to primary amenorrhea and lack of sufficient growth of breast.

Other women referred with primary amenorrhea had small uterus and ovaries or deformities of the uterus and genitals. In some women with primary amenorrhea that have normal karyotype was observed mild mental retardation and behavioral problems and hypothyroidism. For example, one of these women was 20-year-old girl that had hypothyroidism and mental retardation in addition to primary amenorrhea. In addition, in the sonography, her uterus and ovaries were reported small and were reported very high serum hormones of FSH and LH. It

should be noted that the sister of this woman who had Turner syndrome, died in the age of 17. Also two of these women had congenital malformations of cleft lip and palate that one of them had chromosome 1Y (Mixed Gonadal Dysgenesis), but the other one was normal in terms of karyotype.

In a study of women with secondary amenorrhea of 24 women, 22 (91.8%) had normal karyotype, XX46 and 2 females (8.2%) of the women had abnormal karyotype (Table 2). These women had Monosomy chromosomal mosaicism X ((8), XXX 47 / (22), X45) and ((45), XX 46 / (5), X 45), that both of them had clinical symptoms of Turner syndrome (Fig 4) Of the 24 women who had secondary amenorrhea, 8 persons (33.3%) had experienced early menopause that had high FSH and low AMH.

Table 2. The karyotype results in patients with Secondary amenorrhea

karyotype	No. of cases	Diagnosis	Frequency
46,XX	22	SA	91.8%
45,X/46,XX	1	TS	4.1%
45,X/47,XXX	1	TS	4.1%

SA - Secondary amenorrhea; TS - Turner Syndrome

1. **Mixed Gonadal Dysgenesis**

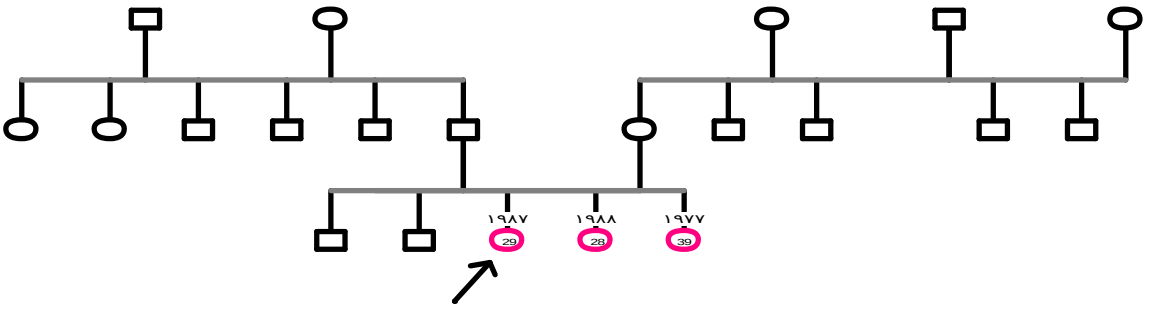

Fig.3. Family tree of three Sisters who had karyotype , XY 46 with symptoms of primary amenorrhea, vaginal blind and testis inguinal

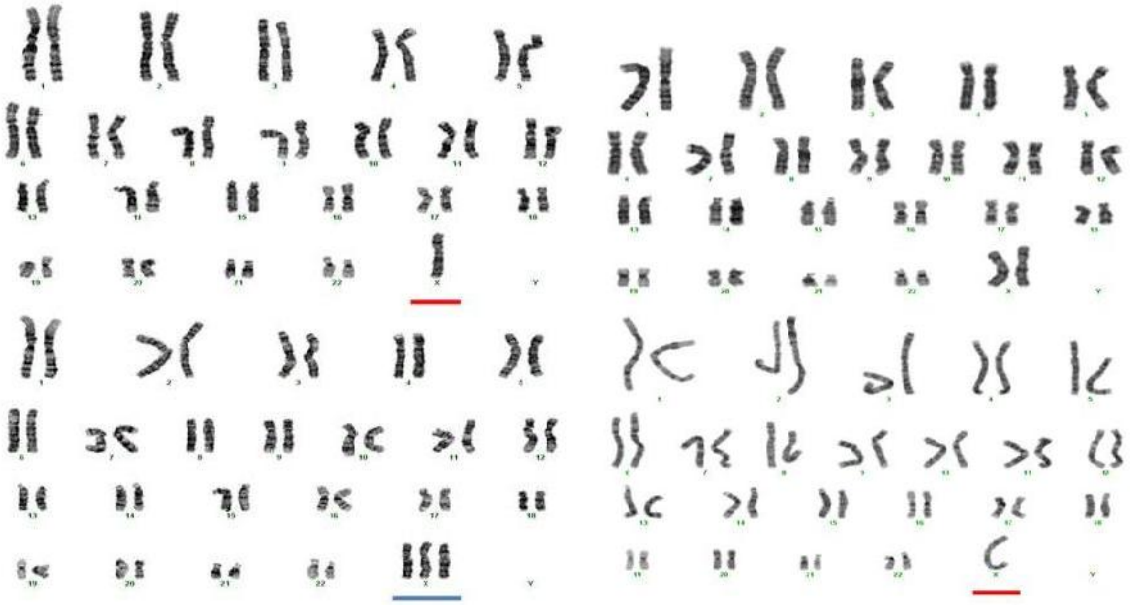

Fig.4. A: Karyotype: 46, XX [45] / 45, X [5]: 50 cells were screened. Two cell lines were observed. 45 cells (90%) were Normal 46, XX and 5 cells (10%) of cells were 45, X. /B: Karyotype: 45, X [22] / 47, XXX [8]: 30 cells were screened. Two different cell lines were observed. One cell line was 45, X (73%) and the other cell line was 47, XXX (27%).

Table3. Chromosomal abnormalities detected in cases with primary amenorrhea in different studies

karyotype	Present study	Butnariu et al (3)	Wong et al (23)	Kong et al (10)	Vijayalaksmi et al (21)	Kalavathi et al (9)	Ramirez et al (6)	Safaei et al(1)
Frequency of abnormal karyotype (number of cases)	**19.76% (17)**	**54.56% (269)**	**24.5 % (58)**	**58.8 % (10)**	**27.8% (39)**	**25.82% (220)**	**36.7% (96)**	**20% (44)**
X chromosome	**41.17% (7)**	**82.15% (221)**	**50% (29)**	**20% (2)**	**74% (29)**	**45.45% (100)**	**89.58% (92)**	**52.27% (23)**

(homogeneous/mosaics) aneuploidies								
X chromosome unbalanced structural abnormalities	**11.76% (2)**	**8.17% (22)**	**12.06 % (7)**	**50% (5)**	**8.69% (3)**	**27.27% (60)**	**4.16% (4)**	**15.90% (7)**
Marker chromosome	**-**	**-**	**1.72 % (1)**	**-**	**-**	**-**	**-**	**-**
Mosaics X/XY and variants	**-**	**3.34% (9)**	**1.72 % (1)**	**-**	**-**	**3.63% (8)**	**-**	**4.54% (2)**
46,XY	**35.29% (6)**	**5.20 % (14)**	**8.4% (20)**	**30% (3)**	**17.9% (7)**	**23.63% (52)**	**7.85% (3)**	**27.27% (12)**
Other anomalies	**11.76% (2)**	**1.11% (3)**	**-**	**-**	**-**	**7.23%**	**-**	**-**

In the previous conducted studies the most common causes of amenorrhea was Turner syndrome, which has been reported in various forms including: 45, X (45%), 45, X / 46, XX (13%), 45, X / 46, X, i (Xq) (8%), (46, X, i (Xq (7%) and 45 X / 46XY (7%).

Although in most cases there is a relationship between these reported karyotype and patients of Turner syndrome but it must be remembered that only based on phenotypic characteristics associated with Turner cannot predict definitely the karyotype of Turner syndrome.

After Monosomy of chromosome X, the most frequent abnormal karyotype (6 – cases ,35.29%) in women with primary amenorrhea, was reported the monosomy karyotype of chromosome Y (, XY 46). These patients had different clinical symptoms such as Gene mixed sexual glands dysfunction, vaginal blind and primary amenorrhea and lack of maturing secondary sexual characteristics.

In previous studies, the frequency of male karyotype for women with primary amenorrhea has been reported from 3.3% percent to 13.7% that results obtained in this study are higher than reported cases in previous studies.

The last group of abnormalities identified in patients with primary amenorrhea, are non-balanced structural abnormalities (2 cases-11.76%) and a balanced structural abnormalities (1Case -5.8%).

In patients with secondary amenorrhea, women with normal karyotype showed higher frequency(91.8%) that can be said most cases of incidence secondary amenorrhea In them is due to glands disorders of the hypothalamus and pituitary [3;2]. Frequency of chromosomal abnormalities in women with secondary amenorrhea was observed 8.2% (2cases of 24 cases).

The percentage of frequency of chromosomal abnormalities in women with secondary amenorrhea of 3.8% to 16.32% has been reported very different. The obtained results in our study were matched like studies with frequency of abnormal karyotype (9/9%) [9]and the frequency (7.08%) observed in another study [10].

In contrast the percentage of frequency of abnormal karyotype in women with secondary amenorrhea in other similar studies with a frequency between 13.3% and 44.4% [11; 12; 13; 4; 14], have reported higher than the results of our study (table 4).

Table 4. Chromosomal abnormalities detected in cases with secondary amenorrhea in different studies

Karyotype results	Present study	Butnariu et al(3)	Wong et al (23)	Lin et al (11)	Rajangam et al(16)	Kalavathi et al (9)	Daevi et al(5)	Optiz et al(15)
No. of Cases	**24**	**38**	**312**	**18**	**245**	**127**	**30**	**15**
46,XX	**22**	**31**	**281**	**10**	**215**	**118**	**26**	**10**
		(81.57%)	**(90.1%)**	**(55.6%)**	**(87.75%)**	**(92.91%)**	**(86.7%)**	**(66.7%)**
Abnormal karyotype	**-**	**7**	**31**	**8**	**40**	**9**	**4**	**5**
		(18.42%)	**(9.9%)**	**(44.4%)**	**(16.32%)**	**(7.08%)**	**(13.3%)**	**(33.3%)**
45,X	**-**	**-**	**5**	**3**	**-**	**1**	**-**	**-**
			(1.6%)	**(16.6%)**		**(0.79%)**		
X monosomy mosaic	**2**	**7**	**11**	**2**	**20**	**8**	**4**	**-**
		(18.42%)	**(3.5%)**	**(11.1%)**	**(8.32)**	**(6.77%)**	**(13.3%)**	
46, del (Xq)	**-**	**-**	**6**	**-**	**-**	**5**	**1**	**-**
			(1.9%)			**(4.23%)**	**(3.3%)**	
46,X,i(Xq)	**-**	**-**	**1**	**-**	**7**	**3**	**1**	**-**
			(0.3%)		**(2.85%)**	**(2.54%)**	**(3.3%)**	
t (X;A)	**-**	**-**	**2**	**1**	**-**	**-**	**2**	**-**
			(0.6%)	**(5.5%)**			**(6.6%)**	
47,XXX	**-**	**-**	**3**	**2**	**3**	**1**	**-**	**-**
			(1%)	**(11.1%)**	**(7.5%)**	**(0.79%)**		
46,XX/47,XXX	**-**	**-**	**3**	**-**	**10**	**-**	**-**	**-**
			(1%)		**(4.65%)**			

Finally in the cytogenetic and clinical evaluation of all patients with primary and secondary amenorrhea in this study (19 cases-17.27%) (12cases -10.9%) had of these women with Turner Syndrome or Turner mosaicism (Table 1 and 2). These patients developed Turner syndrome due to errors occurred during mitotic divisions after fertilization, that are leading to

the removal or absence of the long arm of chromosome X (Xq13-q28)[15].

According to this hypothesis, patients with long arm isochromosome X and or monosomy of short arm of chromosome X with clinical signs such as short stature, Infantilism and sexual glands disorders are along with other congenital abnormalities. On the other hand, patients with X chromosome elimination especially in area (Xq13-q26), who have primary amenorrhea that is due to ovarian disorder [15;16].

In fact, many genes that are essential in the development and or ovarian function, on the long arm of the X chromosome. Inactivation center of X chromosome turns off in the rearrangement of chromosomal in the region Xq13. It is possible that X rearrangements and autosome do not create the interference in the genes that affect ovarian function, but these rearrangements can be due to effect of place cause to change the expression [17]. Existence of isochromosom Xq suggests the increase the risk of developing to hypothyroidism and inflammatory bowel disease [16;18].

Patients with monosomy of Y chromosome have been reported with different clinical symptoms, that in this study were the patients with mixed sexual glands disorders that the best advice for these patients is the removal of these sexual glands to prevent them from getting to Gonadoblastom because these are people with increased risk of developing to Gonadoblastom [19;18].

Furthermore, although patients with trisomy or X chromosome mosaicism do not seem that have clear clinical signs for cytogenetic studies, but should know that chromosome study of these people can lead to a better understanding of the cause of occurrence the amenorrhea In the these people.

On the other hand chromosomal abnormalities in patients with secondary amenorrhea in this study had a lower frequency (2 cases-8.3%), which are nearly similar to other studies which indicate this matter that the various factors other than chromosomal abnormalities can be involved in this matter. In the cases of primary and secondary amenorrhea balanced and lopsided numerical and structural changes of sex chromosome and chromosomal mosaic show a significant relationship with the development of the ovaries and Gonadogenes.

Therefore, in this study also like other previous conducted studies the relationship between

primary and secondary amenorrhea and Chromosomal anomalies were confirmed ($p < 0.05$) that due to the low number of studied women and lack of the breadth of selection criteria of these women, chromosome study the women with primary and secondary amenorrhea, with larger sample sizes and inclusion criteria to more specialized study as a significant cause of infertility leads to a better understanding of its causes and offering the related solutions.

Also, in spite of chromosomal abnormalities in patients with primary and secondary amenorrhea, genetic counseling along with hormone therapy, and other treatment methods are essential for fertility.

Although people with Turner syndrome are suffering from primary infertility, clinical and genetically advice can be very important for people with secondary amenorrhea that are prone to early menopause and also people with mixed sexual glands disorders who have increase the risk of developing to Gonadoblastom

Therefore, performing the cytogenetic tests and genetic counseling are recommended before any action. It should also be noted that the information obtained from cytogenetic tests such as chromosomal analysis are important for management of patient, genetic counseling and future plans of individual.

6. REFERENCES

[1] Novak-E. and Berek-J. S. (2007). Berek & Novak's gynecology, Lippincott Williams & Wilkins.

[2] Schorge-J., Schaffer-J. et al. (2008). "Williams gynecology."

[3] Hughes-I. A. (2008). "Disorders of sex development: a new definition and classification." Best Practice & Research Clinical Endocrinology & Metabolism 22 (1): 119-134.

[4] Rajangam-S. and Nanjappa-L. (2007). "Cytogenetic studies in amenorrhea." Saudi medical journal 28 (2): 187-192.

[5] Rosa-R. F. M., Dibi-R. P. et al. (2008). "Amenorrhea and X chromosome abnormalities." Revista Brasileira de Ginecologia e Obstetrícia 30 (10): 511-517.

[6] Cortés-Gutiérrez-E. I., Dávila-Rodríguez-M. I. et al. (2007). "Prevalence of chromosomal aberrations in Mexican women with primary amenorrhoea." Reproductive biomedicine online

15 (4): 463-467.

[7] McDonough-P. G. (1978). "Amenorrhea-etiologic approach to diagnosis." Fertility and sterility 30 (1): 1-15.

[8] Rooney-D. E. (1986). Human cytogenetics: a practical approach, Oxford University Press, USA.

[9] Wong-M. and Lam-S. (2005). "Cytogenetic analysis of patients with primary and secondary amenorrhoea in Hong Kong: retrospective study." Hong Kong Medical Journal 11 (4): 267.

[10] Kalavathi-V., Chandra-N. et al. (2010). "Chromosomal abnormalities in 979 cases of amenorrhea: A review." International Journal of Human Genetics 10 (1-3): 65-69.

[11] Opitz-O.,Hansmann- I. et al. (1983). "Cytogenetic investigation of 103 patients with primary or secondary amenorrhea." Human genetics 65 (1): 46-47.

[12] Lin-J. and Yu-C. (1996). "[Hypergonadotropic secondary amenorrhea: clinical analysis of 126 cases]." Zhonghua fu chan ke za zhi 31 (5): 278-282.

[13] Devi-A. and Benn-P (1999). "X-chromosome abnormalities in women with premature ovarian failure." The Journal of reproductive medicine 44 (4): 321-324.

[14] Butnariu¹-L., Covic¹-M et al. (2011). "Clinical and cytogenetic correlation in primary and secondary amenorrhea: retrospective study on 531 patients." Revista Română de Medicină de Laborator Vol 19 (2/4).

[15] Wieacker-P. (2009). "Genetic aspects of premature ovarian failure." Journal für Reproduktionsmedizin und Endokrinologie-Journal of Reproductive Medicine and Endocrinology 6 (1): 17-18.

[16] Ogata-T., Muroya-K. et al. (2001). "Turner syndrome and Xp deletions: clinical and molecular studies in 47 patients." The Journal of Clinical Endocrinology & Metabolism 86 (11): 5498-5508.

[17] Behjati-F, Hadipour-F. et al., Cytogenetic analysis of Iranian patients in 13 women with premature ovarian failure, Sarim Encyclopedia, Volume I, Number 3, Summer 2012

[18] Sybert-V. P. and McCauley-E. (2004). "Turner's syndrome." New England Journal of Medicine 351 (12): 1227-1238.

[19] Gravholt-C. H., Fedder- J. et al. (2000). "Occurrence of Gonadoblastoma in Females with Turner Syndrome and Y Chromosome Material: A Population Study 1." The Journal of Clinical Endocrinology & Metabolism 85 (9): 3199-3202.

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