

## Cytogenetic Findings of Patients with Amenorrhea in Turkish Population: A Retrospective Study

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**KEYWORDS** Amenorrhea. Chromosomal Abnormalities. Cytogenetics. Menstruation. Secondary Sexual Characteristics

**ABSTRACT** We performed a retrospective study, with the purpose of establishing the frequency of chromosomal anomalies in amenorrhea cases referred to our genetic laboratory from hospitals in the Middle Black Sea of Turkey. In this study, 105 cases with amenorrhea were analysed and evaluated by Department of Medical Biology and Medical Genetics. Karyotype analyses of cases were made from their peripheral blood lymphocytes by standard method. Twenty metaphases had been prepared with GTG banding method for each patient was analyzed. When a mosaic karyotype was found in any case approximately 100 cells were examined. Chromosomal anomaly was found in 15 (14.3%) patients. Chromosome anomalies in 15 cases were as follows; 34% 45,X, 13.2% 46,XY (testicular feminization), 6.6% mosaic 45,X/46,XX, 6.6% 45,X,del(Xq21), 6.6% 45,X,del(Xp)(p11.21)/45,X, 6.6% 45,X/46,X,der(X), 6.6% t(X;6), 6.6% t(X;14), 11.76% 46,X,iX(q)(10)/45,X. The most common chromosomal abnormalities in cases with amenorrhea were monosomy X and different structural abnormalities of X chromosome respectively.

### INTRODUCTION

Primary amenorrhea was defined as the absence of menstruation and secondary sexual characteristics in phenotypic female aged 14 years or older, or aged 16 or older if secondary sexual characteristics were present (Berek, 2007; Safaei et al. 2010). Patients with secondary amenorrhoea had at least one spontaneous bleeding episode, followed by no menstruation for a minimum of 12 months before the age of 40 years (Wong and Lam 2005; Berek, 2007). Among the general population, amenorrhea is detected in 2%–5% of all females of child-bearing age (Safai et al. 2012). The World Health Organization has estimated %15 of the human population as being amenorrhea that accepted the sixth largest major cause of female infertility. The etiology of amenorrhea has been compartmentalized as disorders of the outflow tract/ovary/anterior pituitary/CNS factors and genetic basis (Rajangam and Nanjappa 2007). 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 (Safai et al. 2012). Genetic factors are considered to be “the causal factors” for most of the conditions in the field of Medicine (Rajangam and Nanjappa 2007). Genetic factors could be single gene disorders/ chromosomal or multifactorial. Among them, chromosome abnormalities are mostly contribute to the etiology of amenorrhea (Berek, 2007). It has been reported that the percentage of chromosomal abnormalities in amenorrhea cases varies from 16.21% to 52.36% in different studies (Wong and Lam 2005; Rajangam and Nanjappa 2007; Saatçi et al. 2000; Safaei et al. 2010; Vijayalakshmi et al. 2010; Kalavathi et al. 2010; Yu et al., 2011; Butnariu et al. 2011).

This retrospective study was undertaken to determine the frequency and type of chromosomal abnormalities in patients with amenorrhoea who live in the Middle Black Sea region of Turkey.

### MATERIAL AND METHOD

The study subjects included cases with amenorrhea (n=105) who referred for chromosomal analysis to the Department of Medical Biology and Genetics, Faculty of Medicine, Ondokuz Mayis University, in the city of Samsun, in the Middle Black Sea coast of Turkey. Primary amenorrhea was defined as the absence of menstruation and secondary sexual charac-

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teristics in phenotypic women aged 14 years older or aged 16 years older if secondary characteristics were present. Patients with secondary amenorrhea had at least one spontaneous bleeding episode, followed by no menstruation for a minimum of 12 months or before the age of 24 years. These cases were referred to our cytogenetic laboratory between 2000 and 2010 years. During presentation, their age ranged from 11 to 32 years with a mean of  $19.429 \pm 5.072$  years. All cases signed informed voluntary consent for this study. Detailed pedigree analysis, clinical evaluation and clinical reports were obtained from all subjects. Chromosomal analysis of all cases were performed by routine lymphocyte culture (Moorhead et al., 1960; Seabright 1971). Minimum 20 metaphases examined for each case. If an abnormal or a mosaic karyotype was found in any case after first analyses, further cells (up to 100) were examined. The karyotypic analysis was performed using Olympus Bx51 microscope (Olympus, Tokyo, Japan) and images were captured with a CCD camera using image analysis system (Cytovision). Karyotypes were described by using ISCN 2009 standard nomenclature of human chromosomes (Shaffer et al., 2009). X-chromatin analysis was made from buccal epithelial cells (Guard, 1959). In addition, fluorescence in-situ hybridization (FISH) analysis was performed in a case. FISH was performed according to manufacturer's specifications. Tel Xq Spectrum Red and Spectrum Green, Sentromer X Spectrum Aqua, LSI Androgen Receptor Spectrum Red were used. Probes were denatured at 73°C for 5 minutes. Following overnight hybridization at 37°C, post hybridization washes were performed and the slides were air-dried in darkness. The slides were counterstained using DAPI (4'-6'-diamidino-2-phenylindole) and stored at -20°C in the dark. The slides were analyzed using an Olympus BX61 fluorescence microscope (Olympus, Tokyo, Japan) and images were captured with

a CCD camera using image analysis system (Applied Imaging, Newcastle, UK). At least 50 metaphase and 50 interphase nuclei were analyzed for each probe. Physical examination of all cases was performed to identify any secondary sexual characteristics and other clinical features by the departments of gynecology and obstetrics.

## RESULTS

In this retrospective study we examined 105 cases with amenorrhea. Primary amenorrhea was identified in 89 (85%) cases and secondary amenorrhea was identified in 16 (15%) cases. The mean age of cases was  $19.429 \pm 5.072$ . The karyotype results revealed that %85.7 of cases with normal chromosome composition (n=90) and 14.3% of cases (n=15) have a chromosomal abnormality (Table 1). Thirty-four percent of cases had complete monosomy 45,X (n=5), 13.2% of them had male karyotype (testicular feminization) (n=2), 13.2% had mosaic genotype (n=2), 13.2% had X chromosome translocations (n=2) and 26.4% of them had structural X chromosome anomalies (n=4). Karyotypic distribution, USG findings, seconder sex characters and hormones of cases were shown in Table 2. In a case which was karyotyped 45,X[97]/46,XX,der(X)dup(Xq) del(Xp)[3] we performed fluorescence in-situ hybridization (FISH) analysis and found mos 45,X/46,XX.ish der(X),dup(p11.2qter)(LSIAR,DXZ1)x2, del(X)(p22.3pter)(subtel-) (Table 3) (Fig. 1).

**Table 1: Karyotypic distribution of cases with amenorrhoea**

Number of cases	105 (100%)	Abnormal karyotype n(%)	15 (14.3%)
Numerical aberrations n(%)			6 (5.7%)
Structural aberrations n(%)			9 (8.6%)
Normal karyotype n(%)			90 (85.7%)

**Table 3: The detailed break points of structural chromosomal abnormality.**

Karyotype
45,X,del(Xq21)(pter→q21: )
45,X[30] 45,X,del(Xp)(p11.21)(qter→p22: ) [70]
45,X[97]/46,XX,der(X)dup(X) (qter→p22.3: : p11.2→qter)del(X)(p11.2→qter)[3]FISH analysis: mos 45,X/46,XX.ish der(X),dup(p11.2qter)(LSIAR,DXZ1) x2, del(X)(p22.3pter)(subtel-)
46,XderX,t(X;6)(q25;q16)(Xpter→q25: : 6q16→6qter;6pter→6q16: : Xq25→Xqter)
46,XderX,t(X;14)(q13;q32)(Xpter→Xq13: : 14q32→14qter;14pter→14q32: : Xq13→Xqter)
46,X,i(X)(qter→q10: : q10→qter)
45,X[40]/ 46,X,i(X)(qter→q10: : q10→qter) [10]

**Table 2: Karyotypic distribution, USG findings, secondary sex characteristics and hormones values of cases**

<i>Karyotype</i>	<i>n (%)</i>	<i>Xchromatin</i>	<i>USG findings</i>	<i>Body height (cm)</i>	<i>Secondary sexual development</i>	<i>Hormones(FSH: mIU/ml, LH: mIU/ml, E2: pmol)</i>	
45, X (Turner Send) n=5 (33%)	45,X	1 (6.6%)	negative	Uterus and ovaries not observed	Short=1.47 cm	No	FSH: 56.69† LH: 29.59† E2: 15.59 Prolactin: 16.02
	45,X	1 (6.6%)	negative	Uterus was small and ovaries not observed	Short =1.55 cm	No	FSH: 149† LH: 35†.3 E2: <10 Prolactin: 21.5
	45,X	1 (6.6%)	negative	Uterus and ovaries not observed	Short =1.47 cm	No	FSH: 7.1 LH: 4.55↓ E2: 27 Prolactin: 10.06 TSH: 0.552 ST3: 4.400† ST4: 1.400
	45,X	1 (6.6%)	negative	Uterus and ovaries not observed	Short =1.45 cm	No	FSH: 6.21 LH: 3.2 E2: 26.9 Prolactin: 18
	45,X	1 (6.6%)	negative	Uterus and ovaries not observed	Short =1.50 cm	No	FSH: 4.32 LH: 2.2↓ E2: <10 Prolactin: 15 TSH: 3.45 ST3: 3.60 ST4: 1.63
45,X[93]/46,XX[7]	1 (6.6%)	negative	Normal USG	Normal=1.65 cm	normal	FSH: 29.59† LH: 11.12† E2: 15 † TSH: 2.41 ST4: 0.87	
46,XY (Testicular Fem.) n=2 (13.2%)	46,XY (TF)	1 (6.6%)	negative	Uterus and ovaries not observed	Normal	poor	FSH: 43.8† TSH: 1.31 E2: 55 Prolactin: 7.45 17-OH Progesterone: 0.45↓ DHEA-Sulphate: 211
	46,XY (TF)	1 (6.6%)	negative	Uterus and ovaries were small	Normal	poor	FSH: 29.8† TSH: 8.21 E2: 55 Prolactin: 9.63
45,X,del(Xq21)	1(6.6%)	-	Ovaries were small	Short =1.50 cm	normal	FSH: 76.89† LH: 39.69† E2: 25.59 Prolactin: 16.05	
45,X,del(Xp)[p11.21] [70]/ 45,X[30]	1(6.6%)	-	Uterus and ovaries were small, vagen was hypopasic	Short =1.50 cm	poor	FSH: 23.5† LH: 7.66 E2: <10 Prolactin: 7.14 DHEA- Sulphate: 111 TSH: 1.7 ST3: 3.89 ST4: 1.24	
45,X[97]/46,XX,der(X)dup(Xq) del(Xp)[3]	1(6.6%)	% 10	Right ovary was multicystic	Short =1.50 cm	poor	FSH: 82.3† LH: 41.8† TSH: 41† Prolactin: 9.29	
46,XderX ,t(X;6)(q25;q16)	1(6.6%)	-	Uterus were small	Short =1.34 cm	No	FSH: 95.88† LH: 37.34† E2: 14.69 TSH: 2.72 ST4: 1.03	
46,XderX, t(X;14)(q13;q32)	1(6.6%)	-	Normal USG and right ovary was multicystic	-	normal	FSH: 75.6† LH: 100.5 E2: 83 Prolactin: 16.93	
46,X,iX(q)(10)	1(6.6%)	-	Uterus was such as tape and ovaries not observed	Short =1.53 cm	No	TSH: 3.5 ST3: 2.86 ST4: 1.25 17-OH Progesterone: 0.71	
46,X,iX(q)(10)[60]/45,X[40]	1(6.6%)	-	Uterus was rudimenter, ovaries not observed	Short =1.47 cm	No	FSH: 13.74 LH: 13.06 E2: 99 TSH: 2.7 ST4: 1.1	
<b>Total</b>	<b>15(100%)</b>						

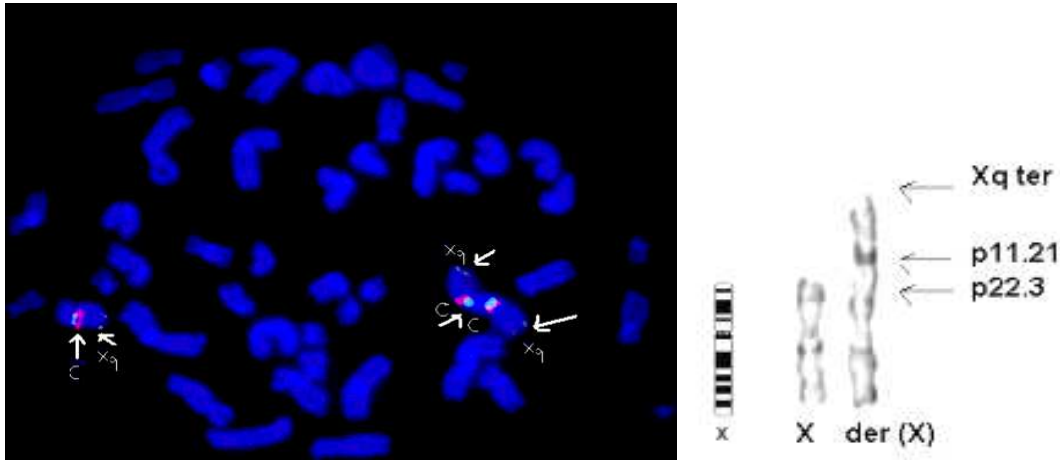


Fig. 1. Chromosomal abnormality in one patient with 45,X[97]/46,XX,der(X)dup(X) (qter→p22.3: : p11.2→qter)del(X)(p11.2→qter)[3] FISH analysis: mos 45,X/46,XX,ish der(X),dup(p11.2qter)(LSIAR,DXZ1) x2, del(X)(p22.3pter)(subtel-)

## DISCUSSION

There are many reasons to cause amenorrhea, chromosomal abnormalities are the most important causes of amenorrhea. Presence of chromosomal abnormality may also affects subsequent management (Safaei et al. 2010). The aim of this study to bring out the cytogenetic findings in cases with primary and secondary amenorrhea in Middle Black Sea Region of Turkey. Several cytogenetic studies had been performed to understand frequency and kind of chromosomal abnormalities in cases with primary amenorrhea (Wong and Lam 2005; Rajangam and Nanjappa 2007; Saatçi et al. 2000; Safaei et al. 2010; Vijayalakshmi et al. 2010; Kalavathi et al. 2010; Yu et al. 2011; Butnariu et al. 2011) (Table 4). In some studies it has been reported that the percentage of chromosomal abnormalities varies from 15.9% to 63.3% cases in amenorrhea until 2005 (Wong and Lam 2005; Joseph and Thomas 1982; Mulye et al. 1983; Ghalib et al. 1988; Ten et al. 1990; Park and Kang 1999). However after 2005 this percentage determined as follows 16.21% to 52.36 % (Wong and Lam 2005; Rajangam and Nanjappa 2007; Saatçi et al. 2008; Safaei et al. 2010; Vijayalakshmi et al. 2010; Kalavathi et al. 2010; Yu et al. 2011; Butnariu et al. 2011).

In the present study, 14.3 % (15/105) of the cases who referred to our laboratory for primary amenorrhea had chromosomal abnormali-

ties. Chromosome aberrations that determined in 15 cases confirmed as follows; 34% 45,X, 13.2% 46,XY (Testicular Feminization), 6.6% mosaic 45,X/46,XX, 6.6% 45,X,del(Xq21), 6.6% 45,X,del(Xp)(p11.21)/45,X, 6.6% 45,X/46,X,der(X), 6.6% t(X;6), 6.6% t(X;14), 11.76% 46,X,iX(q)(10)/45,X. However, chromosomal abnormality in cases with amenorrhea had been reported as 23.55 % in Indian (Kalavathi et al. 2010) and as 47.06% in Chinese (Yu et al. 2011), as 20% in Iranian (Safaei et al. 2010), as 52.36% in Romania (Butnariu et al. 2011). The variation, between the present and the previous studies may be due to the regional differences of patients included for analyses.

Monosomy X is the typical karyotype of Turner's syndrome. Present study results showed that 15 % of the primary amenorrhea cases have X chromosomes abnormalities. The karyotypes of 4.8 % cases was found as pure Turner Syndrome and 0.95% cases was found as 45,X mosaic karyotype. So, our study results were similar to previous studies (Wong and Lam 2005; Rajangam et al. 2007; Saatçi et al. 2008; Kalavathi et al. 2010; Butnariu et al. 2011). Non-disjunction and anaphase lag that to 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 primary amenorrhea cases who had X chromosome monosomy were short stature. And they have also some hormonal changes and absence

**Table 4: Chromosomal abnormalities detected in patients with amenorrhea in different studies.**

Different Studies	Present Study	Butnariu et al., 2011[9]	Yu et al., 2011[8]	India 1979-2004 (24 years)	India 1998-2006 (17 years)	Iran 2005-2008 (3 years)	Turkey 1995-2007 (12 years)	India 1973-2005 (32 years)	Hong Kong 1991-2002 (11 years)
Study population	Turkey 2000-2011 (11 years)	Romania 1985-2009 (24 years)	China 2007-2009 (2 years)	India 1979-2004 (24 years)	India 1998-2006 (17 years)	Iran 2005-2008 (3 years)	Turkey 1995-2007 (12 years)	India 1973-2005 (32 years)	Hong Kong 1991-2002 (11 years)
Study period	105	531	340	981	140	220	165	865	549
Number of cases (n)	90 (85.7 %)	253 (47.64)	180 (52.94 %)	750 (76.45 %)	101 (72.15 %)	176 (80%)	130 (78 %)	663 (76.65%)	460 (83.79 %)
Normal Karyotype	15 (14.3 %)	278 (52.36 %)	160 (47.06 %)	231 (23.55 %)	39 (27.85 %)	44 (20%)	35 (22 %)	202 (23.35%)	89 (16.21 %)
Abnormal karyotype	5 (4.8 %)	137 (25.80 %)	30 (8.83 %)	52 (5.3 %)	11 (7.85 %)	19 (8.64 %)	12 (7.27 %)	39 (4.50 %)	19 (3.46 %)
45,X (Turner Send.)	1 (0.95%)	80 (15.06 %)	9 (2.66 %)	30 (3.05 %)	7 (5 %)	3 (1.37 %)	2 (1.21 %)	32 (3.69%)	26 (4.73 %)
Mosaic Turner	2 (1.9 %)	14 (2.64%)	4 (1.18 %)	53 (5.40%)	7 (5 %)	12 (5.46 %)	8 (4.84 %)	63 (7.29 %)	20 (3.65 %)
46,XY (Testicular Fem.)	-	-	1 (0.29 %)	-	3 (2.14 %)	-	-	1 (0.12 %)	1 (0.18 %)
Marker Chromosome	2 (1.9 %)	12 (2.27%)	18 (5.29%)	31 (3.17%)	4 (2.86 %)	5 (2.28 %)	5 (3.03 %)	-	2 (0.37 %)
Isochromosome	2 (1.9 %)	-	1 (0.3%)	1 (0.10 %)	1 (0.72 %)	1 (0.45 %)	-	8 (0.93 %)	4 (0.73 %)
t(X;A)	3 (2.85 %)	35 (6.59%)	97 (28.51 %)	64 (6.53 %)	6 (4.28 %)	4 (1.8 %)	8 (4.85 %)	59 (6.82 %)	17 (3.09 %)
Others	-	-	-	-	-	-	-	-	-

of secondary sex characters too. The role of sex chromosome in the initiation and maintenance of normal menstruation needs no emphasis. The integrity of a critical area in the X chromosome (q13q26) is essential for normal ovarian function. The integrity for normal ovarian function the (q13q26) area of X chromosome is essential.

In two cases (1.9 %), male chromosome constitution 46,XY karyotype were detected. Many etiological factors can cause abnormal female fetal development leading to 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 (Hughes et al. 2008). All cases with pure gonadal dysgenesis in this study had normal stature. However their FSH was increased and also their seconder sex characters were poor. It had been reported that the incidences of gonadoblastomas, dysgerminomas, and yolk sac tumors varies from 30% to 75% for all females with pure gonadal dysgenesis and 7% to 10% for Turner Syndromes (Behtash and Karimi 2007; Jorgensen et al. 2010). Because of the high risk of neoplastic transformation it is generally been advised that dysgenetic gonads should be surgically removed as soon as diagnosed.

The i(Xq) was present in mosaic or non-mosaic form and similar to individuals with Turner syndrome in the study. It is not suprising because the classical mechanism for isochromosome formation, centromere misadvising, would result in an i(Xq) without Xp. Our results are nearly accordance to previous studies that they have poor seconder sex characters and short stature (Table 4).

In females with balanced X; autosome translocations, the break point position of X chromosome can influence phenotypic outcome. X; autosome translocatios were determined as 1.9% percent in this study (n=2) [46,XderX,t(X;6) and 46,XderX,t(X;14)]. Our results were inconsistent with previous studies. The pattern of X chromosome inactivation is usually associated with an abnormal phenotype and in about 95% of balanced t(X;A), normal X chromosome is inactivated in all cells (Yu et al. 2011). It may be explained of the critical region in Xq26 causes many genes involving premature ovarian failure 1 (*POF1*) to escape inactivation which leading to characteristic phenotypes. However, further study is needed to explain the role of auto-

some X chromosome translocation and it's relation with primary amenorrhea (Vijayalakshmi et al. 2010).

The cases with structural anomalies of the X chromosome, such as deletions, isochromosomes, rings and Xq segmental duplications all showed primary amenorrhea and almost gonadal dysgenesis. It has been pointed that about out 95% of females with deletion in Xp11 showed primary or secondary amenorrhea, but females with deletion in Xp22.3-Xpter had normal menstruation. The critical growth developmental gene *SHOX* was located at Xp11-Xp22.7 region (Yu et al. 2011; Binder 2011). *SHOX* deficiency is a frequent cause of short stature. In accordance with this information cases with deletion or breakpoints in Xp11.1-11.4 region presented short stature in our study. During genetic counseling, it should be informed that, women with mosaic sex chromosome anomalies could be get pregnant. Assisted reproductive strategy has become successful in cases with mosaic Turner syndrome. Because of the risk of gonadal malignancy for cases with XY, it is generally advised that the dysgenetic gonads may be removed by surgically as soon as diagnosed (Safaei et al. 2010).

In this study we presented cytogenetic findings in cases with primary and secondary amenorrhea in Middle Black Sea Region of Turkey. A larger geographical study in Turkey is recommended. Consequently, a significant number of amenorrhea patients had chromosomal anomalies, thus early cytogenetic investigation is prudent to guide further management.

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