Clinical and cytogenetic correlation in primary and secondary amenorrhea: retrospective study on 531 patients

Corelaţii clinice şi citogenetice în amenoreea primară şi secundară: studiu retrospectiv

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Abstract

Primary amenorrhea (PA, absence of menarche) and secondary amenorrhea (SA, the cessation of menstruation in women who were previously menstruating), have many causes including hypothalamic and pituitary disorders, gonadal dysgenesis and utero-vaginal malformations. We performed a retrospective study, with the purpose of establishing the frequency and the type of chromosomal abnormalities, in 531 patients with PA and SA who were clinically and cytogenetically evaluated (1985-2009) in Iaşi Medical Genetics Center.Primary amenorrhea (PA) was identified in 493 (92.84%) patients. X chromatin test, used as a screening test, was abnormal in 201 cases (40.8%) and normal in 292 cases (59.2%). The karyotype was normal in 224 cases (45.43%) and abnormal in 269 (54.56%) patients; the most frequent abnormality detected was X chromosome monosomy, homogeneous (137 cases – 27.78%) or mosaic (80 cases – 16.22%). Other 22 cases (4.46%) had X chromosome structural unbalanced abnormalities (homogeneous or in mosaic). One particular group, represented by 23 patients with PA, had a Y chromosome cell line and the final diagnosis was: pure gonadal dysgenesis (8 cases), CAIS (6 cases), mixed gonadal dysgenesis (4 cases) and true hermaphroditism (5 cases). Other 7 patients presented X trisomy (4 cases) and structural chromosomal abnormalities (3 cases).Secondary amenorrhea (SA) was identified in 38 (7.15%) patients. The X chromatin test and karyotype was normal in 31 of cases (81.57%) and abnormal in 7 cases (18.42%) having X monosomy mosaics. Our results were similar with other reported studies and attest the importance of cytogenetic investigations in the etiologic diagnosis of amenorrhea.

Keywords: *amenorrhea, karyotype, X chromatin test.*

Rezumat

Amenoreea Primară (AP, absenţa menarhei) şi Amenoreea secundară (AS, dispariţia menstruaţiei la o

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femeie care a avut anterior cicluri menstruale), pot avea cauze multiple: hipotalamo-hipofizare, disgenezii gonadice şi malformaţii utero-vaginale. Scopul acestui studiu retrospectiv a fost stabilirea frecvenţei şi a tipului de anomalii cromozomiale, în cazul a 531 paciente cu AP şi AS, care au fost evaluate clinic şi citogenetic (1985-2009) în cadrul Centrului de Genetică Medicală Iaşi. Amenoreea primară (AP) a fost prezentă la 493 (92.84%) de paciente. Testul cromatinei X, folosit ca test de screening, a fost anormal în 201 cazuri (40.8%) şi normal în 292 cazuri (59.2%). Cariotipul a fost normal în 224 cazuri (45.43%) şi anormal în 269 cazuri (54.56%); cea mai frecventă anomalie decelată a fost monosomia X, omogenă (137 cazuri – 27.78%) sau în mozaic (80 cazuri – 16.22%). Alte 22 cazuri (4.46%) au prezentat o anomalie structurală neechilibrată a cromozomului X (omogenă sau în mozaic). Un grup particular a fost reprezentat de 23 de paciente cu AP, care prezentau o linie celulară cu un cromozom Y şi diagnosticul final: disgenezie gonadică pură (8 cazuri), CAIS (6 cazuri), disgenezie gonadică mixtă (4 cazuri) şi hermafroditism adevărat (5 cazuri). Alte 7 paciente au prezentat trisomie X (4 cazuri) şi alte anomalii cromozomiale de structură (3 cazuri). Amenoreea secundară (SA) a fost prezentă la 38 (7.15%) de paciente. Testul cromatinei X şi cariotipul au fost normale în 31 cazuri (81.57%) şi anormale în 7 cazuri (18.42%), care au prezentat o monosomie X în mozaic. Rezultatele noastre, similare cu cele prezentate în alte studii, atestă importanţa investigaţiilor citogenetice pentru diagnosticul etiologic al amenoreei.

Cuvinte cheie: *amenoree, cariotip, cromatina X.*

Introduction

Amenorrhea (A) is the absence or abnormal cessation of the menses (1). It can be classified as primary amenorrhea (PA) – the failure of menses to occur by the age of 16, or secondary amenorrhea (SA) – in which the menses appeared at puberty but subsequently ceased (1). The incidence of PA in the United States is less than 1% and the prevalence of SA is 5-7%; no evidence indicates that the prevalence of amenorrhea varies according to national origin or ethnic group (2).

The main causes of PA are: pituitary / hypothalamic disorders (27.8 %); gonadal /ovarian disorders (50.4%); outflow tract (uterine-vaginal) abnormalities (21.8 %) (1). Thus, half of PA cases are determined by the gonadal / ovarian disorders, frequently produced by abnormal sex chromosomes (2). Usually, gonadal dysgenesis is produced by X chromosome monosomy (45,X), typical of Turner Syndrome (TS), but amenorrhea is also seen in 47,XXX trisomy, in pure 46,XX gonadal dysgenesis and 46,XY gonadal dysgenesis (Swyer syndrome), or in the intersex disorders (Complete androgen insensitivity syndrome-CAIS; mixed gonadal dysgenesis- MGD) (2). The majority of cases of secondary amenorrhea (SA) are not produced by chromosomal abnormalities, like 45, X or 47,XXX.(2).

The aim of study

The aim of our study was to estimate, by a retrospective analysis, the frequency and the type of chromosomal abnormalities, in patients with PA and SA. All our patients were clinically evaluated and cytogenetically investigated in The Genetic Medical Centre of Iaşi (GMC), (between 1985 and 2009). The cytogenetic analyses were performed in the Cytogenetics Laboratory, at the „Gr. T. Popa" University of Medicine and Pharmacy, Iaşi.

Materials and methods

Patients

From January 1985 to January 2009 we performed a clinical, paraclinical and cytogenetic evaluation (by X chromatin test and karyotype) of 531 patients with primary amenorrhea (PA) and early secondary amenorrhea (SA) (5 to 10 years after menarche) with ages between 13 years and 45 years; 493 patients (92.84%) had primary amenorrhea and 38 patients (7.15%) had secondary amenorrhea.

The clinical examination included anthropometric measurements (weight, height, cranial perimeter, biacromial and bitrochanteric diameter), evaluation of pubertal development

(based on Tanner's stages I-V, the age of the beginning of menarche, presence or absence of menstrual cycle, breasts development and presence of axillary and pubic hair) and evaluation of presence of typical features of Turner syndrome (short stature, cubitus valgus, craniofacial dysmorphism).

Cytogenetic investigations

The cytogenetic investigations were X-chromatin test and chromosomal analysis using G-banded metaphases.

The X - chromatin (Barr body) was analyzed on buccal mucosa smear, fixed in a mixture of absolute ethanol and glacial acetic acid (3:1), and stained with a carbol-fuchsin solution (3). In every case were analyzed 300 cells in order to establish the presence, the number and the size of the Barr bodies. The X - chromatin test was considered abnormal: in all patients with absent Barr body (negative test); in cases of patients with low percentage (<10%) of Barr bodies, suggesting a chromosomal mosaicism; in patients with number and size abnormalities of Barr bodies (≠ 1μm), suggesting X chromosome numerical and structural abnormalities (isochromosomes, deletions) (3).

Chromosomal analysis was based on a short-term culture of activated T- lymphocytes stimulated with phytohemagglutinin (Moorhed method ameliorated in our laboratory) and contains the following stages: sampling 3-5 ml of peripheral blood in sodium heparin vacutainer tube; blood cells (T-lymphocites) were cultured (37° for 72 hours) on RPMI 1640 medium (9 ml), supplemented with fetal calf serum (1 ml), L - glutamine (0.1 ml), phytohemagglutinin and antibiotics (penicillin and streptomycin); then, the culture was treated with a hypotonic solution, fixed, and dropped onto a microscope slide; the slides were stained and examined on optical microscope, direct or after application of G- banding (after trypsin treatment) (450-550 bands). The metaphases were analyzed with a Nikon microscope and images were captured with an automated image analysis system (Cytovision, Applied Imaging). For each case a minimum 32 metaphases were analyzed, thus permitting the identification of chromosomal mosaics in more than 90% of cases. One cell line was validated when we found at least 3 cells with the same karyotype. When we detected more than 2 cell lines, the number of analyzed cell was increased to 64 or 96.

Results

1. The cytogenetic results in patients with primary and early secondary amenorrhea

a) In the sample group of 493 (92.84%) patients with primary amenorrhea (PA) the results of X chromatin test was abnormal in 201 cases (40.8%) [Barr test negative - 154; positive <10% -21; 2 Barr bodies- 16; Barr body > 1 μm - 10] and normal (positive >20%) in 292 cases (59.2%). The karyotype in patients with PA was abnormal in 269 cases (54.56%). We identified a X chromosome homogeneous monosomy, 45,X in 137 cases (27.78%), a X chromosome monosomy mosaicism in 105 cases (21.29%), and other anomalies in 27 cases (5.47%) (*Table 1*).

The karyotype was normal in 224 cases (45.43%), with the PA having another etiology than chromosomal abnormalities (hypothalamic or pituitary disorders, utero-vaginal abnormalities).

The most frequent abnormalities discovered in patients with PA (*Table 1*) were X chromosome homogeneous monosomy, 45,X – typical of Turner Syndrome (TS) (137 cases – 27.78%) and different forms of X chromosome monosomy mosaicism (105 cases -21.29%). The frequency of X chromosome homogeneous monosomy in cases of patients with PA was more statistically significant than the frequency of X chromosome monosomy mosaicism and X chromosome structural abnormalities ($\chi 2(2.269) = 71.40$: $p < 0.01$; $p< 0.05$). In 80 cases (16.22%) we discovered a mosaic aneuploidy having mostly the 45,X cell line. Other 19 cases

Table 1. Chromosomal abnormalities in 269 patients with primary amenorrhea

Type of chromosomal abnormalities	X Chromatin*	Diagnosis	No. of cases	Frequency % *****
X chromosome homogenous monosomy			137	27.78
45,X	negative	TS	136	27.58
45,X, inv(5)(p14;q11)**	negative	TS	1	0.20
X chromosome monosomy mosaicism			80	16.22
45,X/46,XX	positive<10% (21 cases) positive (45cases)	TS	66	13.38
45,X/46,XX/47,XXX	positive-2B	TS	7	1.61
45,X/47,XXX	positive-2B	TS	6	1.21
45,X,inv(9)(p13;q21)/47,XXX,inv(9)(p13;q21)***	positive-2B	TS	1	0.20
X chromosome unbalanced structural abnormalities			3	0.60
46,X,i(Xq)	positive B>	TS	2	0.40
46,X,del(Xp)(p11.2-p11.4)	positive	TS	1	0.20
Aneuploid mosaics with X chromosome unbalanced structural abnormalities			19	3.85
45,X/46,X,i(Xq)	positive B>	TS	8	1.62
45,X/ 46,X,i (Xq)/46,XX	positive	TS	1	0.20
46,X,i(Xq)/46,XX	positive	TS	1	0.20
45,X/46,X,r(X)	positive	TS	3	0.60
45,X/46,XX/46,X,r(X)/ 47,XXX	positive	TS	2	0.40
46,X,r(X)/46,XX	positive	TS	1	0.20
45,X/45,X,+min/46,XX,+min/47,XX,r(X) ****	positive	TS	1	0.20
45,X/46,X,del(X)(q22→qter)	positive	TS	2	0.40
46,XY sex-reversal syndrome	negative	PGD/CAIS	14	2.83
Aneuploid mosaics with one cell line with Y chromosome			9	1.82
45,X/47,XXY	Positive	MGD	1	0.20
45,X/46,XY	negative	MGD	2	0.40
45,X/46,X,dic(Y)	negative	MGD	1	0.20
45,X/46,XX/47,XXY	Positive	TH	1	0.20
45,X/46,XX/46,XY	Positive	TH	1	0.20
46,XX/46,XY	Positive	TH	1	0.20
46,XX/47,XXY	Positive	TH	2	0.40
Trisomy X			4	0.80
47,XXX	positive-2B	TXS	2	0.40
46,XX/47,XXX	Positive	TXS	2	0.40
Balanced X-autosom translocations			1	
46,X,ins (1;X)(q21.1;q11.3→21.2)	Positive	PA	1	0.20
Balanced autosomal structural abnormalities			1	0.20
46,XX,ins(4;6)(q31;q32)	Positive	PA	1	0.20
Others			1	0.40
46,XX,add(1)(q21)	positive	PA	1	0.20
Total			269	54.56

TS - Turner Syndrome; PA - Primary Amenorrhea; MGD - Mixed Gonadal Dysgenesis; TH - True Hermaphroditism; PGD - Pure Gonadal Dysgenesis; TXS - Triple X Syndrome; CAIS – Complete Androgen Insensitivity Syndrome; B- Barr body
* X chromatin: abnormal = negative; positive <10%; 2B - two Barr bodies; B> - Barr body >1μm; ** It is possible that inv(5)(p14;q11 was inherited from one of the parents; *** inv(9)(p13;q21) is a polymorphic chromosome variant. **** The origin of minute chromosome could not be identified. ***** percentage was in relation to the total number of patients with PA (493 cases)

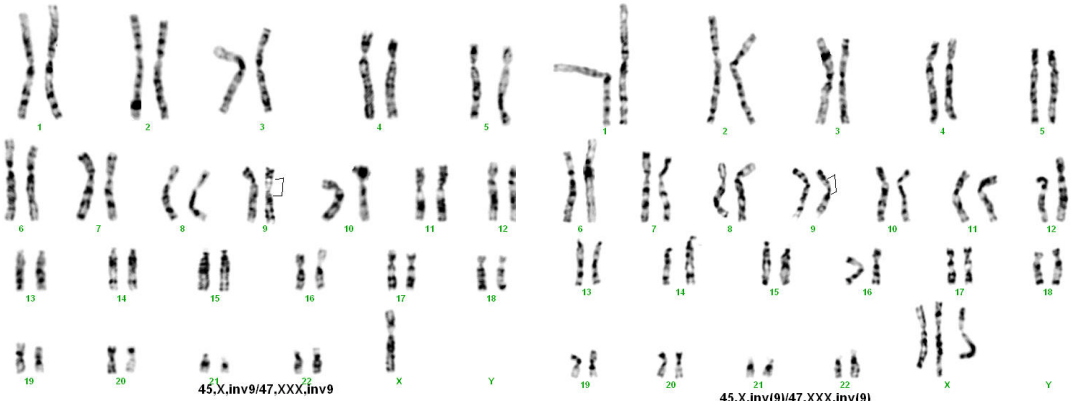

Figure. 1. Case B.S. 9,11 years: 45,X,inv(9)(p13;q12)/47,XXX, inv(9)(p13;q12)

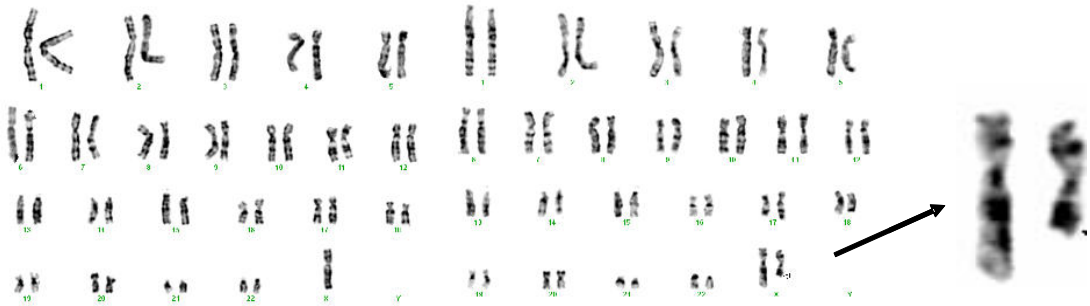

Figure 2. Case G.A. 40 years: 45,X/46,X,del (X)(q22→qter)

(3.85%) with a clinical diagnosis of TS presented a mosaicism with 1 cell line with X monosomy and the second line with an unbalanced structural abnormality of X chromosome: the most frequent abnormality detected was isochromosomes Xq (10 cases- 2.02%), followed by ring X chromosomes (7 cases – 1.41%) and Xq deletions (2 cases -0.40%) (*Table 1, and Figures 1, 2, 3 and 4*).

In only 3 cases (2 i(Xq) and 1 del(Xp)) we identified a homogeneous unbalanced structural abnormality of X chromosome (*Table 1*).

One particular group was represented by 23 patients with PA having one cell line with normal / abnormal Y chromosome. The karyotype result was 46,XY in 14 cases (2.83%) which had the following clinical diagnosis: pure gonadal dysgenesis (8 cases) and CAIS (Complete Androgen Insensitivity Syndrome, 6 cases); in other 9 cases (1.82%) with mosaics having one cell line 45,X and/or 46,XX and the other cell line with Y chromosome, the diagnosis was: mixed gonadal dysgenesis (4 cases) and true hermaphroditism (5 cases) (*Table 1*).

In 4 cases (0.80%) trisomy X was detected (homogeneous - 47,XXX or in mosaic 46,XX/47,XXX); in other 2 cases (0.40%) was identified a balanced structural chromosomal abnormality (*Table 1*): between the X chromosome and an autosome - 46,XX,ins(1;X)(q21.1;q11.3-21.2) and between two autosomes - 46,XX, ins(4;6)(q31;q33). One patient with PA had an unbalanced structural chromosomal abnormality: 46,XX,add(1)(q21) (0.20%); the origin of the supplementary chromosome 1 band could not been identified.

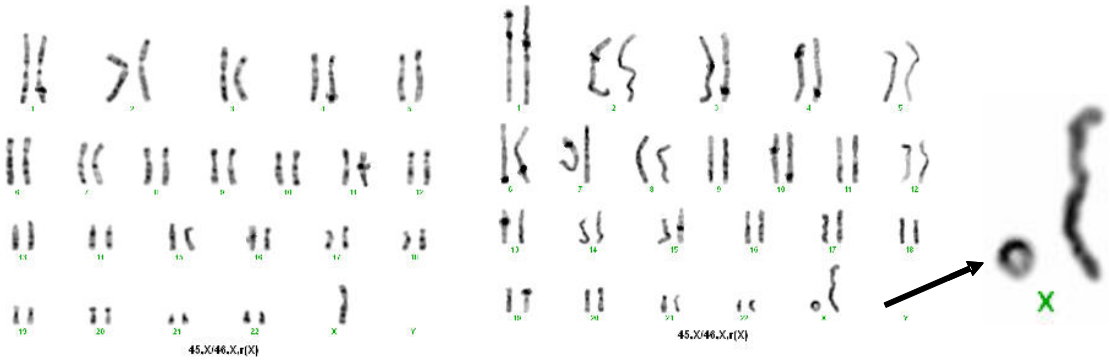

Figure.3 Case 3, B.V.,20 years: 45,X/46,X,r(X)

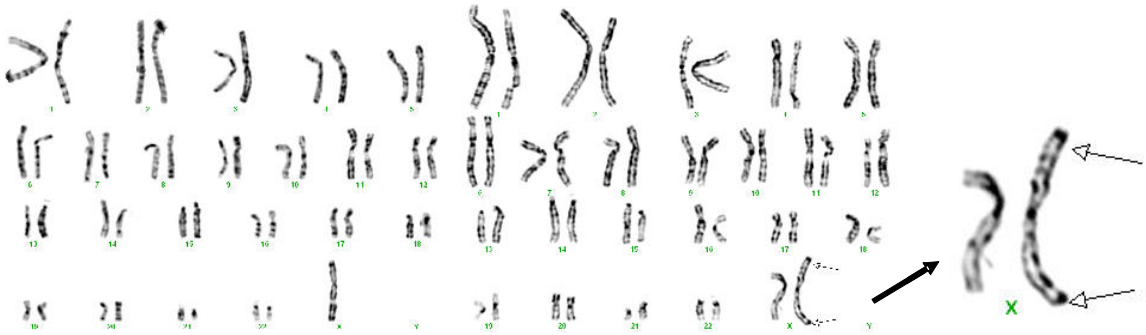

Figure.4. Case 4 C.G. 19,6 years: 45,X/46,X,i(Xq)

b) In patients with secondary amenorrhea (SA) (38 cases – 7.15%) the X chromatin test was normal in 31 of cases (81.57%) and abnormal (positive, but with low percentage of Barr body, or two Barr bodies) in 7 cases (18.42%). Chromosomal analysis was normal (46,XX) in the majority of cases (31 patients), but in 7 cases, having abnormal X chromatin test, we detected X monosomy mosaics: 45,X/46,XX (4 cases), 45,X/47,XXX (2 cases) and 45,X/46,XX/47,XXX (1 case); 6 patients with SA were diagnosed with Turner Syndrome and 1 patients (with 45,X/47,XXX karyotype) presented SA determined by Premature Ovarian Failure (POF) (*Table 2*).

2. Clinical and cytogenetic correlation in patients with primary and early secondary amenorrhea.

Most patients with X chromosome homogenous monosomy, 45,X (137 cases, 27.78%) presented clinical features of Turner Syndrome (short stature, primary amenorrhea, poor development of secondary sex characteristics, pterygium colli, cubitus valgus, craniofacial dysmorphism) and high plasma levels of FSH and LH (hypergonadotropic hypogonadism) (*Table 1*). In 25 cases (18.24%) cardiac defects were detected and 20 patients (14.59%) presented renal malformations. In cases of patients with primary amenorrhea (PA) and mosaic or structural X-chromosome abnormalities (108 cases, 21.90%), the clinical features of TS were present, but inconstant. The frequency of X chromosome homogeneous monosomy (27.78%, 137 cases) in case of patients with Turner syndrome was more statistically significant than the frequency of X chromosome monosomy mosaicism and X chromosome structural abnormalities ($\chi 2(2.245 = 8.31: p < 0.01; p< 0.05$).

Table 2. The karyotype results in patients with Secondary Amenorrhea (SA)

Karyotype	X chromatin*	Diagnosis	No. of cases	Frequency %
46,XX	positive	SA	31	81.57 %
45,X/46,XX	positive <10%	TS	4	10.52%
45,X/47,XXX	positive – 2B	TS	1	2.63%
45,X/47,XXX	positive – 2B	SA/POF	1	2.63%
45,X/46,XX/47,XXX	positive - 2B	TS	1	0.35%

SA: Secondary Amenorrhea; TS: Turner Syndrome; POF: Premature Ovarian Failure; B: Barr body
* X chromatin: abnormal= negative or positive <10%; 2B- two Barr bodies.

The second frequent group of chromosomal abnormalities detected in patients with PA was the presence of Y chromosome (23 cases), but the clinical features were variable. We detected 14 patients (2.83%) with PA and 46,XY karyotype (*Table 1*). Chromosomal analysis confirmed the clinical diagnosis of CAIS in 6 cases (1.21%) who presented female phenotype, the absence of the fallopian tubes, uterus and cervix, and intraabdominal testis. In 8 cases (1.62%) with PA the patients had Müllerian duct derivatives, sexual infantilism and low plasma testosterone levels. The diagnosis was 46,XY pure gonadal dysgenesis (Swyer syndrome).

Other five patients (1.01%) with PA presented a chromosomal mosaic with one of the cell lines containing a Y chromosome (*Table 1*): 46,XX/47,XXY (2 cases), 46,XX/46,XY (1 case), 45,X/46,XX/47,XXY (1 case), 45,X/46,XX/46,XY (1 case). All these patients presented PA, clitoris hypertrophy, normal breast development, uterus, an ovotestis and a contralateral dysgenetic gonad (detected by laparoscopic gonad biopsy). The clinical diagnosis was: true hermaphroditism (TH). Four patients (0.80%) with PA and karyotypes 45,X/47,XXY (1 case), 45,X/46,XY (2 cases) and 45,X/46,X,dic(Y) (1 case) were diagnosed with mixed gonadal dysgenesis (MGD); they presented poor development of secondary sex characteristics and variable TS stigmata: short stature, ponderal hypotrophy, craniofacial dysmorphism, pterigyum colli, cubitus valgus and pigmented nevi. Ultrasound examination and subsequent exploratory laparoscopy showed the absence of the internal genital organs, as well as dysgenetic testis on one side and an ovarian tissue by the other side.

The last group of 7 patients with PA and chromosomal abnormalities presented various abnormal karyotypes, the association with PA being somewhat surprising. Four (0.80%) of these patients were diagnosed with triple X syndrome: 47,XXX (2 cases – 0.40%) and 46,XX/47,XXX (2 cases – 0.40%) (*Table 1*). They presented a nonspecific craniofacial dysmorphism (down-slanting palpebral fissures, epicanthus), poor development of secondary sex characteristics, short stature, and normal intellectual development. Other three patients with PA without any special phenotypic features, presented structural chromosomal abnormalities: one insertion between X chromosome and an autosome - 46,XX,ins(1;X)(q21.1;q11.3-21.2); one insertion between two autosomes - 46,XX, ins(4;6)(q31;q33) and one insertion with indeterminate origin on chromosome 1: 46,XX, add(1)(q21) (*Table 1*).

In patients with secondary amenorrhea (38 cases), chromosomal analysis (*Table 2*) identified various chromosomal mosaics in 7 cases; in 6 cases the clinical diagnosis was Turner Syndrome, in one case (karyotype 45,X/47,XXX) the patient presented SA determined by Premature Ovarian Failure (POF).

Table 3. Chromosomal abnormalities detected in cases with primary amenorrhea in different studies

Karyotype results	Present study	Wong et al (4)	Kong et al (6)	Vijayalaksmi et al (7)	Kalavathi et al. (8)	Ramirez et al (9)	Safaei et al (10)
Frequency of abnormal karyotype (number of cases)	**54.56% (269)**	**24.5% (58)**	**58.8% (10)**	**27.8% (39)**	**25.82% (220)**	**36.7% (96)**	**20% (44)**
X chromosome (homogeneous/mosaics) aneuploidies *	**82.15% (221)**	50% (29)	20% (2)	74% (29)	45.45% (100)	89.58% (92)	52.27% (23)
X chromosome unbalanced structural abnormalities	**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	**5.20 % (14)**	8.4% (20)	30% (3)	17.9% (7)	23.63% (52)	7.85% (3)	27.27% (12)
Other anomalies	**1.11% (3)**			-	7.23%	-	-

*were included 45,X and 47,XXX- -homogeneous and mosaics

This patient (37 years) presented: menarche at 16 years, irregular menstrual cycles and SA at 26 years; stature 161 cm; almost normal secondary sex characteristics, uterus and ovaries with low dimensions, high plasma levels of FSH and LH; in this case we considered that secondary amenorrhea has been produced by premature ovarian follicular depletion.

Discussions

1. Cytogenetic evaluation of patients with PA and SA

In 201 cases with PA (40.8%) we identified a strong correlation between the results of Barr test and karyotype.

The high percentage of chromosomal abnormalities (54.56%) detected on our patients with PA suggested the major role of chromosomal abnormalities in the abnormal gonadal development and function, as well as the importance of genetic investigation of patients with PA and SA. The frequency of abnormal karyotypes has been reported to vary between 15.9% and 63.3% among women with primary amenorrhea (Wong and Lam, 2005) (4), with the majority falling between 24% and 46% (Cortes - Gutierrez et al, 2007) (5). Our result (54.56%) was in accordance to the results obtained by Kong et al, 2007 - 58.8% (6), and higher than in other studies: Wong et Lam, 2005 – 24.50% (4) Vijayalaksmi et al, 2010 -27.8% (7), Kalavathi et al, 2010 – 25.82% (8), Ramirez et al, 2000 – 36.7% (9) and Safaei et al, 2010 - 20% (10) (*Table 3*). The differences between the present results and that of the previous studies may be due to the wide variation in patient selection criteria of different studies.

We noticed the high frequency of chromosomal mosaics, with two or more cell lines, in cases with PA: 110/269 (40.9%) and SA: 7/31 (18.42%). The percentage of mosaicism ranged

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

Karyotype results	Present study	Wong et al (4)	Kalavathi et al (8)	Rajangam et al (12)	Daevi et al (17)	Lin et al, (19)	Opitz et al, (21)
No. of cases	**38**	312	127	245	30	18	15
46,XX karyotype	**31 (81.57%)**	281 (90.1%)	118 (92.91%)	215 (87.75%)	26 (86.7%)	10 (55.6%)	10 (66.7%)
Abnormal karyotype	**7 (18.42%)**	31 (9.9%)	9 (7.08%)	40 (16.32%)	4 (13.3%)	8 (44.4%)	5 (33.3%)
45,X	-	5 (1.6%)	1 (0.79%)	-	-	3 (16.6%)	-
X monosomy mosaic	**7 (18.42%)**	11 (3.5%)	8 (6.77%)	20 (8.32)	4 (13.3%)	2 (11.1%)	-
46, del (Xq)	-	6 (1.9%)	5 (4.23%)	-	1 (3.3%)	-	-
46,X,i(Xq)	-	1 (0.3%)	3	7 (2.85%)	1	-	-
t (X;A)	-	2 (0.6%)	-	-	2 (6.6%	1 (5.5%)	
47,XXX	-	3 (1%)	1 (0.79%)	3 (7.5%)	-	2 (11.1%)	
46,XX/47,XXX	-	3 (1%)	-	10 (4.65%)	-		

from 10% to 70% in diferrent studies (10). Our result was similar to other studies: Vijayalaksmi et al, 2010 – 51.2% of cases (7) and Baros et al, 2009 – 33.8% (11). The karyotype was normal in 224 cases with PA (45.43%), the absence of menarche having another etiology than chromosomal abnormalities (hypothalamic or pituitary disorders, or utero-vaginal malformations. In patients with SA the high frequency of normal karyotypes (81.57%) was expected, since SA was a frequent consequence of the hypothalamic or pituitary disorders (1,2). The frequency of chromosomal abnormalities detected in patients with SA was 18,42% (7 cases). The percentage of chromosomal abnormalities reported for SA varies greatly, from 3.8% to 44.4%. Our result was in accordance with others reported in similar studies: Rajangam et al, 2010 – 16.32% (12), Daevi et al, 1999 – 13.3% (13). In other studies the frequency of chromosomal abnormalities was variable: 7.08% (Kalavathi et al, 2010) (8), 9.09% (Wong et Lam, 2005) (4), 44.4% (Lin et al, 1996) (14) and 33.3% (Opitz et al, 1993) (15) (*Table 4*).

We noticed that 239 cases with PA had a total or partial X chromosome monosomy: 137 cases with X chromosome homogeneous monosomy (57.32%); 80 cases (33.47%) with numerical mosaicism; 19 cases (7.94%) with mosaic X structural unbalanced chromosomal abnormalities and 3 cases (1.25%) with homogeneous X chromosome structural abnormalities.

Similar results were reported by Ramirez et al, 2000: X chromosome homogeneous monosomy - 49 cases (52.1%), X chromosome numerical mosaicism - 43 cases (45.74%) and X chromosome unbalanced structural ab-

normalities - 4 cases (4.25%) (9). Other results were significantly higher compared to our result, in cases of X structural unbalanced chromosomal abnormalities: Kalavathi et al, 2010 – 27.27% (8) Wong and Lam, 2005 – 12.06% (4), and Safaei et al, 2007 – 15.9% (10) (*Table 3*). In previous studies the most commonly-occurring karyotypes reported in Turner syndrome were: 45,X (45%), 45,X/46,XX (13%), 45,X/46,X,i(Xq) (8%), 46,X,i(Xq) (7%) and 45,X/46,XY (7%); although some correlation between karyotype and phenotype have been made, phenotypic predictions for a given patient that are based on karyotypic analysis are unreliable in patients with Turner syndrome (16).

The second frequent group of chromosomal abnormalities detected in patients with PA was represented by the homogeneous or in mosaics karyotypes having a cell line with Y chromosome (23 cases – 8.55%). These patients presented different syndromes: 46,XY pure gonadal dysgenesis (8 cases – 2.97%), CAIS (6 cases -2.23%), true hermaphroditism (5 cases -1.85%) and mixed gonadal dysgenesis (4 cases - 1.48%). Male karyotype and mosaics karyotypes having a cell line with Y chromosome were present in a significant percentage of the patients with PA in previous studies ranged from 3.3% to 13.7% (10). Our results were comparable with these studies, and were similar to Vijayalaksmi et al, 2010 who detected 6.42% cases (9 patients) with homogeneous or in mosaics karyotypes having a cell line with Y chromosome: 46,XY (7 cases -5%), 45,X/46,XY (1 case – 0.7%) and 46,XX/46,XY (1 case – 0.7%) (7).

The last group of chromosomal abnormalities detected in patients with PA (7 cases-2.60%) (*Table 1*) was represented by homogeneous or mosaic X trisomy (4 cases), balanced structural chromosomal abnormalities (2 cases – 0.74%): one between X chromosome and an autosome [46,XX,ins(1;X)(q21.1;q11.3-21.2)] and another one between two autosomes [46,XX,ins(4;6)(q31;q33)], and one unbalanced structural chromosomal abnormality: 46,XX, add(1)(q21), with indeterminate origin of chromosome 1 supplementary band.

2. Clinical and cytogenetic types of primary amenorrhea (PA)

The clinical and cytogenetic diagnosis was Turner syndrome in the group of 245 patients (46.14% of all study patients) with PA (239 cases) and SA (6 cases).

The present study confirmed the high frequency of cardiac abnormalities (18.24%) and renal abnormalities (14.59%) detected in patients with Turner Syndrome, but our values were lower than those reported in similar studies. A possible explanation could be an incomplete evaluation of our patients, or a population characteristic. The frequency of cardiac abnormalities detected in patients with Turner syndrome has been reported to vary between 17 to 60% (Sybert and McCaulay, 2004) (17). The results were variable in similar studies: Proprawsky et al, 2009 – 21% (18), Korpal-Szczyrska et al, 2005 – 32.7% (19), Völkl et al, 2005 – 29.9% (20), Saenger, 1996 - 45-65% (21). The frequency of renal abnormalities has been reported to vary between 27 and 65% (17,22). In a similar study, Bilge et al, 2000, detected a renal abnormality in 20% of cases (22).

The cytogenetic evaluation revealed a X chromosome monosomy mosaicism or X chromosome structural abnormalities - homogeneous or in mosaics, in others 108 patients (44.08%) diagnosed with Turner Syndrome (*Table 1 and Table 2*); these patients presented inconstant clinical features of TS. In these cases, the chromosomal abnormalities were produced by postzygotic mitotic errors; the variable clinical features of patients can be explicated by theories of Fraccaro et al. 1977 and Wyss et al, 1982 (23). They postulated that the genes whose absences are responsible for somatic features of TS were located on Xp or Xq (Xq 13-q26). Based on these hypotheses the phenotype of patients with isochromosome (Xq) (and Xp monosomy) can be correlated with short stature and some Turner stigmata

(sexual infantilism, gonadal dysgenesis), and association with other congenital malformations. On the other side, the patients with chromosome X deletions involving „critical region" on Xq (Xq13-Xq26), present primary amenorrhea determined by an ovarian dysgenesis. (23,24). The presence of an isochromosome Xq suggests an increased risk for hypotiroidism and inflammatory bowel diseases and the presence of a ring or marker chromosome confers an increased risk of mental retardation and atypical phenotypic features (17, 24).

The second frequent group of chromosomal abnormalities detected in patients with PA was a homogeneous or in mosaics karyotypes having a cell line with Y chromosome (23 cases, table 1), but the clinical context was variable: CAIS, 46,XY pure gonadal dysgenesis (Swyer syndrome), true hermaphroditism and mixed gonadal dysgenesis. In these cases is recommended prophylactic gonadectomy, because of the high risk (7% to 30%) of gonadoblastoma in the dysgenetic gonads (17,26). Our results underline the necessity of a good collaboration between different specialists and the use of standards and rigorous criteria of diagnosis.

Patients with trisomy X (homogeneous or in mosaics) had uncharacteristic clinical features, certain diagnosis being established by cytogenetic analysis (Barr test and karyotype).

In patients with SA we detected the chromosomal abnormalities in 7 cases (18.42%); our results were similar to other studies (*Table 4*). These findings are not surprising, because many SA cases are generated by the non chromosomal causes.

The presence of a chromosomal abnormality in patients with primary and secondary amenorrhea imposed the genetic counseling, with the presentation of possible therapies (hormonal therapy of substitution) and reproductive options (assisted reproduction). The psychological counseling was very important, because the patients with Turner Syndrome had primary sterility, the patients with secondary amenorrhea had a high risk for premature menopause, and because of the high risk of gonadoblastoma in cases of patients with mixed gonadal dysgenesis.

Conclusions

In conclusion, the present study (undertaken on a large number of patients, with PA and SA) confirms that sex chromosome abnormalities, numerical or structural, homogeneous or in mosaic, represent a major etiologic factor in primary or secondary amenorrhea (54.56% in cases of PA and 18.42% in cases of SA); the majority of patients presented ovarian dysgenesis and hypergonadotropic hypogonadism. Our results were in accordance to other similar studies.

In this context, we emphasize the importance of cytogenetic investigations, the X chromatin test as a cytogenetic screening test and the karyotype for confirmation of the certain diagnosis, in all patients with (primary or secondary) amenorrhea. An early diagnosis is helpful for patient counseling and management.

Abbvrebiations

PA- Primary Amenorrhea
SA- Secondary Amenorrhea
TS- Turner Syndrome
B- Barr body
TSF- Testicular Feminization Syndrome
MGD- Mixed Gonadal Dysgenesis
TH- True Hermaphroditism
PGD- Pure Gonadal Dysgenesis
MPH- Male Pseudohermaphroditism
TXS- Triple X Syndrome

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