Gonadal Function in Turner Syndrome

Função Gonadal na Síndrome de Turner

Márcia ALVES[1], Margarida BASTOS[1], Teresa ALMEIDA SANTOS[2], Francisco CARRILHO[1]
Acta Med Port 2013 Nov-Dec;26(6):655-663

ABSTRACT

Introduction: Turner syndrome is characterized by the absence, total or partial, of one X chromosome in females, being one of the most frequent chromosomal abnormalities. Diagnosis is made by karyotype. Turner syndrome manifestations include primary hypogonadism, before or after puberty (gonadal dysgenesis). The degree and extent of gonadal disfunction are variable.

Objectives: We intended to assess clinical, karyotype, gonadal function and pelvic ultrasound characteristics in women with Turner syndrome.

Material and Methods: Retrospective study of patients with Turner syndrome followed in Endocrinology and Human Reproduction Departments of Hospitais da Universidade de Coimbra - Centro Hospitalar e Universitário de Coimbra, E.P.E. We evaluated the entire sample and considered group 1 (with spontaneous puberty and menarche) and group 2 (without spontaneous puberty). Parameters assessed: age at initial study, puberty (Tanner stages), karyotype, FSH, pelvic ultrasound (initial and after puberty), diagnostic laparoscopy and pubertal induction. Statistical Program: SPSS (20.0).

Results: Global sample: 79 patients, 14.7 ± 6.6 years. No pubertal signs in 57.1%; 67.1% with primary amenorrhea and 6.6% with secondary amenorrhea. Karyotype: X monosomy-37.2%, mosaicism-37.2%, X structural changes-25.6%. Median FSH of 59.5 mIU/mL. Initial ultrasound: normal uterus 34.2%, atrophic uterus 65.8%; normal ovaries 21.6%, atrophic ovaries 78.4%, ovarian follicles in 5.1%. Post-puberty ultrasound: normal uterus 67.9%, atrophic uterus 32.1%; normal ovaries 36.4%, atrophic ovaries 63.6%. Laparoscopy was performed in 16 (20.3%) patients, confirming the sonographic findings. Only two women with induced puberty became pregnant: one spontaneously, interrupted; another by donated oocytes, normal outcome. Group 1 (with spontaneous puberty and menarche): 20 (25.3%) patients, 16.1 ± 8.9 years. Tanner at baseline: M1-22.2%, M2-33.3%, M3-16.7%, M4-16.7%, M5-11.1%. Karyotype: mosaicism-65%, X structural changes-20%, X monosomy-15%. Median FSH of 7 mUI/mL. Initial ultrasound: normal uterus-72.2%, atrophic uterus 27.8%; normal ovaries 63.2%, atrophic ovaries 36.8%. Post-puberty ultrasound: normal uterus 100%; normal ovaries 72.7%, atrophic ovaries 27.3%. Group 2 (without spontaneous puberty): 59 (74.7%) patients, 14.0 ± 5.5 years. Tanner at baseline: M1-69.2%, M2-13.5%, M3-5.8%, M4-3.8%, M5-7.7%. Karyotype: X monosomy-43.9%, X structural changes-28.1% mosaicism-28.1%. Median FSH of 74 mUI/mL. Initial ultrasound: normal uterus 20.4%, atrophic uterus 79.6%; normal ovaries 7.4%, atrophic ovaries 92.6%. Post-puberty ultrasound: normal uterus 60.0%, atrophic uterus 40.0%; normal ovaries 27.3%, atrophic ovaries 72.7%. Pubertal induction at 16.1 ± 4.1 years, with bone age of 12.7 ± 1.6 years. Groups 1 and 2 differ significantly in karyotype ($p = 0.010$), median FSH ($p < 0.001$), and uterine and ovarian dimensions ($p < 0.001$).

Conclusions: Most patients had gonadal dysfunction and needed pubertal induction. Spontaneous puberty with menarche occurred in 25.3% of patients (predominantly mosaics). 43.9% of patients with pubertal induction had X monosomy. These patients fertility is compromised and, in some cases, we should refer to assisted reproductive specialist for pregnancy or fertility preservation.

Keywords: Gonadal Disorders; Menstruation Disturbances; Turner Syndrome.

RESUMO

Introdução: A síndrome de Turner caracteriza-se pela ausência, parcial ou total, de um cromossoma X no sexo feminino, sendo uma das cromossomopatias mais frequentes. O diagnóstico é realizado através do cariótipo e as suas manifestações incluem o hipogonadismo primário, antes ou após a puberdade (disgenesia gonadal). O grau de disfunção e a extensão dos defeitos gonadais são variáveis.

Objectivos: Pretendeu-se avaliar a clínica, cariótipo, função gonadal e características ecográficas do útero e ovários de mulheres com síndrome de Turner.

Material e Métodos: Estudo retrospectivo de doentes com síndrome de Turner, seguidas nos Serviços de Endocrinologia ou Reprodução Humana dos Hospitais da Universidade de Coimbra - Centro Hospitalar e Universitário de Coimbra, E.P.E. Avaliou-se toda a amostra e consideraram-se o grupo 1 (com puberdade e menarca espontânea) e grupo 2 (sem puberdade espontânea). Parâmetros avaliados: idade do estudo inicial, puberdade, cariótipo, FSH, ecografia pélvica inicial e pós-pubertária, celioscopia e indução pubertária. Estudo estatístico: SPSS (20.0).

Resultados: Amostra: 79 doentes, 14,7 ± 6,6 anos. Ausência de sinais pubertários em 57,1%, amenorreia primária 67,1% e secundária 6,6%. Cariótipo: monossomia X-37,2%, mosaico-37,2%, alterações estruturais de X-25,6%. Mediana da FSH 59,5mUI/mL. Ecografia inicial: útero normal-34,2%, atrófico-65,8%; ovários normais-21,6%, atróficos-78,4%, com folículos-5,1%. Ecografia pós-pubertária: útero normal-67,9%, atrófico-32,1%; ovários normais-36,4%, atróficos-63,6%. A laparoscopia realizada em 16 (20,3%) doentes confirmou os achados ecográficos. Duas mulheres com puberdade induzida engravidaram: uma espontaneamente, sem evolução; outra por doação de ovócitos, evolutiva. Grupo 1 (com puberdade e menarca espontânea): 20 (25,3%) doentes, 16,1 ± 8,9 anos. Puberdade na avaliação inicial: M1-22,2%, M2-33,3%, M3-16,7%, M4-16,7%, M5-11,1%. Cariótipo: mosaico-65%, alterações estruturais de X-20%, monossomia X-15%. Mediana da FSH 7 mUI/mL. Ecografia inicial: útero normal-72,2%, atrófico-27,8%; ovários normais-63,2%,

1. Serviço de Endocrinologia, Diabetes e Metabolismo. Centro Hospitalar e Universitário de Coimbra, EPE. Coimbra. Portugal.
2. Serviço de Reprodução Humana. Centro Hospitalar e Universitário de Coimbra, EPE. Coimbra. Portugal.
Recebido: 26 de Janeiro de 2013 - Aceite: 07 de Outubro de 2013

atróficos-36,8%. Ecografia pós-pubertária: útero normal-100%; ovários normais-72,7%, atróficos-27,3%. Grupo 2 (sem puberdade espontânea): 59 (74,7%) doentes, 14,0 ± 5,5 anos. Puberdade na avaliação inicial: M1-69,2%, M2-13,5%, M3-5,8%, M4-3,8%, M5-7,7%. Cariótipo: monossomia X-43,9%, alterações estruturais de X-28,1%, mosaico-28,1%. Mediana da FSH 74 mUI/mL. Ecografia inicial: útero normal-20,4%, atrófico-79,6%; ovários normais-7,4%, atróficos-92,6%. Ecografia pós-pubertária: útero normal-60,0%, atrófico-40,0%; ovários normais-27,3%, atróficos-72,7%. Indução pubertária aos 16,1 ± 4,1 anos com idade óssea 12,7 ± 1,6 anos. Os grupos 1 e 2 diferiram significativamente no cariótipo (p = 0,010), FSH (p < 0,001), dimensões do útero e ovários (p < 0,001).

Conclusões: A maioria das doentes apresentou disfunção gonadal com necessidade de indução pubertária. Ocorreu puberdade e menarca espontâneas em 25,3% das doentes (predomínio de mosaicos). Das doentes com indução pubertária, 43,9% apresentavam monossomia X. A fertilidade destas doentes está comprometida, podendo nalgumas situações recorrer-se a técnicas de procriação medicamente assistida para obter uma gravidez ou preservar a fertilidade.

Palavras-chave: Alterações Gonadais; Distúrbios Menstruais; Síndrome de Turner.

INTRODUCTION

Turner syndrome (TS) is defined by a partial or total absence of one X chromosome in females[1] and represents one of the most common chromosomal abnormalities.[2]

It is present in approximately 1:2,500 to 1:4,000 female newborns.[1] Prenatal prevalence is higher, which means a higher conception rate of foetus with TS.[2] Nevertheless, there is a high intrauterine mortality, especially during the first trimester of pregnancy (with a peak around the 13th week).[3]

TS is associated with a large variety of phenotypic abnormalities, most of them caused by haploinsufficiency of the genes normally expressed by both X chromosomes.[4] Cardinal features include growth retardation with short height, gonadal insufficiency and infertility.[1,4,5]

Diagnosis is obtained by karyotyping. In clinical practice, about half of the patients with TS present a 45,X karyotype (X monosomy), 20 to 30% with a mosaicism (45,X and at least one more cell line) and the remaining display structural abnormalities of one of the X chromosomes.[1,4]

Clinical presentation of TS includes primary hypogonadism, whether occurring before or after puberty (gonadal dysgenesis). The dysfunction degree and the extension of gonadal defects are variable.[6]

During foetal life, a foetus with TS develops a normal number of oocytes, although most of them present with accelerated atresia.[7-9] The cause for this fact is unknown and it is assumed that oocytes without a normal second chromosome X are not viable. Characteristically, ovaries in TS are reduced to small amounts of connective tissue with no follicles or only some atretic follicles.

Nevertheless, up to 30% of the girls with TS present some degree of spontaneous puberty, about 10% achieve menarche and it is estimated that 2-5% are fertile.[10-12] These frequencies are probably undervalued as many women with TS and preserved fertility are not diagnosed.

Hormone replacement therapy must be started at the normal puberty age and must be continued until the age of 50.[4] The dose of oestrogen must be enough to avoid clinical manifestations of hypogonadism and long-term consequences of oestrogen deficit, particularly the loss of bone mass.[6]

This study's objective was to assess clinical context, karyotype, gonadal function and uterine and gonadal ultrasound signs in females with TS.

MATERIAL AND METHODS

A retrospective study has been carried out using clinical records (manuscripts and computerised) of patients with TS, followed at the Endocrinology and/or Gynaecology departments from *Centro Hospitalar e Universitário de Coimbra*, *HUC-CHUC*.

The following parameters were evaluated: spontaneous (with or without menarche) or induced puberty, age of the patient when the initial clinical assessment took place, karyotype, Tanner pubertal stages (at the initial clinical assessment and after puberty), pre-pubertal FSH levels (assay obtained at initial assessment or before pubertal induction), gynaecological ultrasound (initial and after pubertal induction), coelioscopy and/or gynaecological surgery.

Patients were subdivided in two groups: group 1, patients presenting spontaneous puberty and menarche; and group 2, without spontaneous puberty.

Both groups were assessed and compared with each other, regarding: patient's age when the initial clinical assessment took place, karyotype, Tanner puberty stages (at the first clinical assessment and after puberty), pre-pubertal FSH levels and gynaecological ultrasound (at initial assessment and after puberty).

In group 2, pubertal induction, chronological and bone age at the time of induction and oestrogen type were assessed.

Statistical analysis was carried out using SPSS 20.0 software.

RESULTS

Seventy-nine patients with TS were assessed. From these, 30 (38.0%) presented an X monosomy, 29 (36.7%) presented a mosaic pattern with intact X chromosomes and 20 (25.3%) presented structural chromosomal abnormalities (Table 1).

Mean age at the first clinical assessment was 14.7 ± 6.6 years. At that time, signs of pubertal development were absent in 57.1% of the patients, while 18.6% presented a breast bud (Tanner stage 2), 8.6% were in stage 3, 7.1% in stage 4 and 8.6% in stage 5; eight patients (10.1%) presented external genitalia atrophy (Table 1).

Pre-pubertal FSH median level was 59.50 (1:29.3 - 3:104.8) mIU/mL.

Table 1 – Total sample characteristics and frequencies.

Sample characteristics		*n*	Percentage (%)
Puberty	Spontaneous (with menarche)	20	25.3
	Induced	59	74.7
	Total	**79**	**100.0**
Karyotype	X monosomy	30	38.0
	Mosaic with intact X	29	36.7
	X structural abnormalities	20	25.3
	Total	**79**	**100.0**
Initial pubertal stage *	M 1	40	57.1
	M 2	13	18.6
	M 3	6	8.6
	M 4	5	7.1
	M 5	6	8.6
	Total	**70**	**100.0**
External genitalia atrophy		8	10.1
Pre-pubertal uterus	Normal	25	34.2
	Atrophic	48	65.8
	Total	**73**	**100.0**
Pre-pubertal ovaries	Normal	16	21.6
	Atrophic	58	78.4
	Total	**74**	**100.0**
Coelioscopy – Uterus	Normal	3	23.1
	Atrophic	10	76.9
	Total	**13**	**100.0**
Coelioscopy – Ovaries	Normal	1	7.7
	Atrophic	12	92.3
	Total	**13**	**100.0**
Bilateral gonadectomy		4	5.1
Total hysterectomy		2	2.5
Final pubertal stage *	M 1	1	1.4
	M 2	3	4.3
	M 3	3	4.3
	M 4	15	21.7
	M 5	47	68.1
	Total	**69**	**100.0**
Post-pubertal uterus	Normal	38	67.9
	Atrophic	18	32.1
	Total	**56**	**100.0**
Post-pubertal ovaries	Normal	20	36.4
	Atrophic	35	63.6
	Total	**55**	**100.0**

*: M1 – pre-pubertal stage; M2 – breast bud; M3 – wider breast and areola; M4 – areola and papilla extend beyond breast contour; M5 – breast and areola at the same level; adult morphology.

Initial gynaecological ultrasound revealed a normal uterus in 34.2% of the patients which was atrophic in the remaining 65.8%; the ovaries had a normal appearance in 21.6% of the patients and were atrophic in 78.4% (Table 1); four patients presented visible ovarian follicles on ultrasound imaging.

A diagnostic laparoscopy (coelioscopy) has been performed in 16 patients with TS. Mean age when this procedure was performed was 17.1 ± 4.9 years; laparoscopic results revealed the presence of a normal uterus in 23.1% of the patients, atrophic in the remaining (76.9%); ovaries were normal in only one of the patients and atrophic in 12 (92.3%) (Table 1).

Six patients were submitted to gynaecological surgery (7.6% of all the patients) (Table 1), with a mean age at the moment of surgery of 19.5 ± 5.5 years. Four of these patients were submitted to bilateral gonadectomy due to the presence of SRY mosaic material and two other patients were submitted to a total hysterectomy, also due to the presence of SRY mosaic material in a karyotype with a mosaicism pattern and one of the patients presented an ovarian dysgerminoma.

The end of puberty, determined by clinical or ultrasound assessment, occurred on average, at 19.4 years. Ultrasound re-evaluation carried out in 56 patients revealed a normal uterus in 67.9%, atrophic in 32.1%; normal ovaries in 36.4% and atrophic ovaries in 63.6% of the patients (Table 1).

The average period of follow-up since the first assessment until the end of puberty was 6.3 ± 3.8 years.

Spontaneous puberty with menarche occurred in 20 patients (25.3%). These were considered as group 1 (*n* = 20). Fifty-nine patients with TS with an indication for pubertal induction (74.7% of the total) were considered as group 2 (*n* = 59).

In group 1, most of the patients presented a mosaic karyotype with an intact X chromosome (65%); however, there were 20% of the patients with chromosomal structural abnormalities and the remaining 15% with an X monosomy. In group 2, most of the patients (45.8%) presented an X monosomy, while 27.1% of the patients presented a karyotype with chromosomal structural abnormalities and 27.1% with a mosaic karyotype and an intact X chromosome (Table 2).

Karyotypes with X chromosome structural abnormalities and their relation with the different groups were assessed (Table 3). Spontaneous puberty was observed in two

Table 2 – Group 1 (with spontaneous puberty and menarche) and 2 (with induced puberty characteristics).

Characteristics		Group 1		Group 2	
		n	%	*n*	%
Karyotype	X monosomy	3	15.0	27	45.8
	Mosaic with intact X	13	65.0	16	27.1
	X structural abnormalities	4	20.0	16	27.1
	Total	**20**	**100**	**59**	**100**
Initial pubertal stage *	M 1	4	22.2	36	69.2
	M 2	6	33.3	7	13.5
	M 3	3	16.7	3	5.8
	M 4	3	16.7	2	3.8
	M 5	2	11.1	4	7.7
	Total	**18**	**100,0**	**52**	**100.0**
Initial ultrasound – Uterus	Normal	13	72.2	11	20.4
	Atrophic	5	27.8	43	79.6
	Total	**18**	**100.0**	**54**	**100.0**
Initial ultrasound – Ovaries	Normal	12	63.2	4	7.4
	Atrophic	7	36.8	50	92.6
	Total	**19**	**100**	**54**	**100.0**
Final pubertal stage *	M 1	0	0.0	1	1.9
	M 2	1	6.2	2	3.8
	M 3	0	0.0	3	5.7
	M 4	1	6.2	14	26.4
	M 5	14	87.5	33	62.3
	Total	**16**	**100,0**	**53**	**100.0**
Post-pubertal ultrasound Uterus	Normal	11	100.0	27	60.0
	Atrophic	0	0.0	18	40.0
	Total	**11**	**100.0**	**45**	**100.0**
Post-pubertal ultrasound Ovaries	Normal	8	72.7	12	27.3
	Atrophic	3	27.3	32	72.7
	Total	**11**	**100.0**	**44**	**100.0**

*: M1 – pre-pubertal stage; M2 – breast bud; M3 – wider breast and areola; M4 – areola and papilla extend beyond breast contour; M5 – breast and areola at the same level; adult morphology.

patients with an X chromosome short arm deletion, in one patient with a mosaic and an X chromosome short arm deletion and in another patient with a mosaic with an X chromosome short arm deletion. Spontaneous menarche did not occur in any of the patients with an isochromosome or X chromosome long arm deletion or with a ring X chromosome.

Comparing both groups, mosaic frequency with an X long arm isochromosome was significantly higher in group 2 (p = 0.015).

We also assessed the different cell lines in the mosaic karyotypes (45,X, 46,XX, 47,XXX and 46,XY). We did not observe any significant difference between the groups regarding the expression of the different cell lines, although the 46,XX cell line was the most frequent in group 1 patients.

Among mosaic patients with spontaneous puberty (group 1), 92.3% presented a 46,XX cell line (n = 12), 7.7% presented a 47,XXX cell line (n = 1) and another 7.7% presented a 46,XY cell line (n = 1).

Comparing both groups, as regards the quantitative expression of the different cell lines present in peripheral blood, we observed a higher expression of 46,XX in group 1 and a smaller expression of 45,X, 47,XXX and 46,XY lines. We observed differences in the quantitative distribution of cell lines in mosaic patients with spontaneous menarche, while spontaneous menarche was more commonly affected in mosaic patients with a higher expression of the 46,XX cell line (p = 0.011) which was lower in the 45,X cell line patients (p = 0.011) (Table 4).

At the first clinical assessment, the mean age in group 1 was 16.1 ± 8.9 years, while in group 2 it was 14.0 ± 5.5 years. At that time, 22.2% of the patients in group 1 did not present pubertal signs (Tanner stage 1), while 33.3% presented in stage 2, 16.7% in stage 3, 16.7% in stage 4 and 11.1% in stage 5. In group 2, 69.2% of the patients presented no signs of puberty development (stage 1), 13.5% were in stage 2, 5.8% in stage 3, 3.8% in stage 4 and 7.7% in stage 5 (Table 4).

Median FSH levels (pre-pubertal or at first assessment, if menarche had already occurred) were 7 [1:5-3:31] mIU/mL in group 1 and 74 [1:52-3:110] mIU/mL in group 2.

Pre-pubertal ultrasound assessment in group 1 patients showed a normal uterus in 72.2% of the patients and an atrophic uterus in 27.8%; ovaries were normal in 63.2% of the patients and atrophic in 36.8%. We observed an atrophic uterus in 79.6% of group 2 patients and a normal uterus in only 20.4%; ovaries were atrophic in 92.6% and normal in only 7.4% (Table 4).

At pubertal induction, group 2 patients had a chronological mean age of 16.1 ± 4.1 years and mean bone age was 12.7 ± 1.6 years.

In 76.7% of the patients, puberty was induced with oral oestrogen and in the remaining 23.3% with transdermal oestrogen. Progesterone introduction occurred, on average, at 17.2 ± 1.8 years of chronological age, with a mean bone age of 13.5 ± 1.1 years (on average 1.1 years after beginning oestrogen therapy).

After puberty, 87.5% of the patients in group 1 presented in full growth development (stage 5), while in group 2 only 62.3% of the patients were at the same stage (Table 2). The differences between group 1 and 2 regarding pubertal stages were not significant.

Ultrasound re-assessment in group 1 patients, at the end of puberty or at the last clinical assessment, showed

Table 3 – Distribution of karyotypes with X chromosome structural abnormalities.

X Chromosome structural abnormalities	Group 1		Group 2		Total	
	n	%	*n*	%	*N*	%
Mosaic X long arm isochromosome (45.X/46.X.i(Xq))	0	0.0	11	55.0	**11**	**55.0**
X short arm deletion (46.X.del(Xp))	2	10.0	1	5.0	**3**	**15.0**
X long arm isochromosome (46.X.i(Xq))	0	0.0	2	10.0	**2**	**10.0**
Mosaic with an X short arm deletion (45.X/46.X.del(Xq))	1	5.0	0	0.0	**1**	**5.0**
Mosaico com deleção do braço curto do X (45,X/46,X,del(Xp))	1	5.0	0	0.0	**1**	**5.0**
Mosaic with an X short arm deletion (45.X/46.X.del(Xp))	0	0.0	1	5.0	**1**	**5.0**
Mosaic with a ring X chromosome (45.X/46.X.r(X))	0	0.0	1	5.0	**1**	**5.0**
Total	**4**	**20.0**	**16**	**80.0**	**20**	**100.0**

Table 4 – Quantitative expression of the different cell lines in mosaic karyotypes with an intact X chromosome.

Quantitative expression of cell lines in mosaic patterns	Group 1 (%)	Group 2 (%)
45.X	4.0 [1:1.0-3:99.0]	38.7 [1:1.0-3:99.0]
46.XX	96.4 [1:71.3-3:99.0]	46.7 [1:0.9-3:99]
46.XY	1.0 [1:1.0-3:1.0]	70.0 [1:50.0-3:90.0]
47.XXX	2.0 [1:2.0-3:2.0]	3.8 [1:0.5-3:33.3]

a normal uterus in all the patients, normal ovaries in 72.7% and atrophic in 27.3% of the patients. Ultrasound re-assessment in group 2 showed a normal uterus in 60.0% of the patients, an atrophic uterus in 40.0%; normal ovaries in 27.3%, and atrophic ovaries in 72.7% (Table 4).

The mean age at first assessment was higher in group 1 patients (16.1 ± 8.9 years) than in group 2, who presented earlier for medical assessment (at 14.0 ± 5.5 years). However, this difference was not significant using the Mann-Whitney test ($p = 0.587$).

There were significant differences between the groups regarding karyotype ($p = 0.010$), FSH ($p < 0.001$), uterus and ovary dimensions ($p < 0.001$).

The occurrence of two pregnancies, both in patients with TS, submitted to pubertal induction (group 2) is highlighted. One occurred in one patient assessed for amenorrhea at the age of 33, by then with a normal uterus in the initial ultrasound assessment; hormone therapy was only started at the age of 33, when TS diagnosis was established; pregnancy may have been spontaneous at the age of 31, with foetal death *in utero* at 30 weeks, according to the patient. The other pregnancy occurred in a patient with TS diagnosed since the age of 17, with primary amenorrhea and absence of pubertal signs at the initial assessment. Due to a mosaicism with a Y chromosome (45,X/46,XY) she was submitted to bilateral gonadectomy at the age of 18. The patient was submitted to *in vitro* fertilization with oocyte donation at the age of 40, in Spain, resulting in a twin pregnancy, apparently without maternal or foetal complications.

DISCUSSION

We observed in our group of patients with TS a relation between gonadal function, karyotype and pre-pubertal gonadotropin levels. However, the retrospective nature of the study through analysis of medical records data which were not always complete, limited the relevant information and the quality of the obtained results.

It is known that in TS patients, spontaneous pubertal development should be assessed by clinical examination and FSH hormone assay, from the age of 10.[13] Mean age at the first medical assessment at our hospital was delayed, occurring at 14.7 ± 6.6 years, explained by a hospital bias for adult medicine.

The mean age in group 1 patients at the presentation was 16.1 ± 8.9 years, while in group 2 it was 14.0 ± 5.5 years. This difference may be explained by the presence of a spontaneous menarche, which delayed TS diagnosis in group 1 patients. However, the difference between the groups was not significant.

Karyotype proved to be highly predictive of gonadal function, but the fact that assessment was performed in peripheral blood lymphocytes did not allow for detection of low-degree mosaicism.

When using X^2 test, we observed a significant difference ($p = 0.010$) in karyotype distribution (between? spontaneous puberty and induced puberty, respectively). Spontaneous puberty development was more commonly observed in patients with TS and a mosaic karyotype, a result which is in line with previous reports.[10,14-17] Equally, as previously reported, some patients with TS and patients other karyotypes such as an X monosomy, also presented spontaneous puberty with menarche.[10,14,15,17-22]

There is a weak phenotype/genotype correlation in TS with a mosaic karyotype as phenotype variability depends on the different degrees and tissue patterns of mosaicism. In our study, we observed differences regarding quantitative distribution of cell lines in mosaic karyotypes, with spontaneous menarche being more frequent in mosaic patients, in the presence of the 46,XX cell line ($p = 0.011$) and negatively correlated with the 45,X cell lines ($p = 0.011$). These differences are related with the presence of a normal cell line (46,XX).

Normal gonadal development needs the ZFX gene in the X chromosome short arm. Women with an X monosomy, an X long arm isochromosome and short arm deletions commonly present with gonadal dysgenesis due to haploinsufficiency of this gene. Furthermore, some regions in the X chromosome long arm are related with ovarian failure: premature ovarian failure type 1, which covers the Xq26-qter region and premature ovarian failure type 2, which covers the q13,3 - Xq22. Clinically, patients with abnormalities in these regions present primary amenorrhea with hypogonadotropic hypogonadism or secondary amenorrhea with infertility. In our group of patients, the presence of an X long arm isochromosome, even in mosaicism with other cell lines, did not present spontaneous menarche in group 2 patients, what is in line with what has been previously described. The presence of an X long arm isochromosome mosaic in our group of patients seems to determine a lower odds ratio of achieving menarche spontaneously (OR = 0.313; 95% CI: 0.151 – 0.646).

In our group of patients, there was only one ring X chromosome patient, with an indication for pubertal induction due to gonadal dysgenesis.

In our group of patients, X chromosome deletions (long arm or short arm) were associated with variable phenotypes, almost entirely with spontaneous menarche, except one case in which there was a pubertal induction. These results contradict those described in literature, in which short arm deletions, isolated or in mosaic and those that are more distal from the long arm generally present with gonadal dysgenesis. This difference may be due to the absence, in our group of patients, of a deletion of the regions related with gonadal dysfunction.

At the moment of the first assessment, group 1 patients presented mainly in earlier pubertal stages (Tanner stage 1 in 22.2% and stage 2 in 33.3% of the patients), despite a balanced distribution between the different stages. Most of the patients in group 2 were in stage 1 (69.2%). As expected, this difference was significant in the initial pubertal stage ($p = 0.001$).

Ultrasound initial uterine and ovarian characteristics

were significantly different between the groups (Mann-Whitney test ($p < 0.001$). The lack of uterine and ovarian abnormalities was higher in group 1 patients, in line with clinical presentation. Normal or just slightly high pre-pubertal gonadotropin levels, namely FSH, demonstrate a strong association with spontaneous pubertal development and the existence of ovarian follicles.[10,12,14,17,23] In the present study, FSH was determined before the beginning of puberty (spontaneous or induced) or at the moment of the first assessment (if menarche had already occurred) and the median of the global sample was 59.50 [1:29.3 - 3:104.8] mIU/mL. This median was significantly lower in group 1 (7 [1:5-3:31] mIU/mL) than in group 2 patients (74 [1:52-3:110] mIU/mL), which is in line with the observed pubertal differences ($p < 0.001$). Levels of inhibin B and the anti-mullerian hormone have not been taken into consideration, as these were not considered routine investigations in patients with TS and were therefore not available in most cases.

When FSH is clearly high and the clinical signs of puberty are absent, a pubertal induction with oestrogen should be started. The purpose of this induction is to achieve a similar physical and psychological development as the one occurring in a spontaneous puberty and to establish adequate peak bone mass.[24] However, as oestrogen speed up bone epiphyseal fusion, the moment of starting therapy must be coordinated (commonly with growth hormone therapy), aiming to achieve maximum growth potential, without unduly delaying the beginning of puberty.[25]

In group 2 patients, at the moment of pubertal induction, chronological mean age was 16.1 ± 4.1 years and mean bone age was 12.7 ± 1.6 years. Recent studies demonstrate that starting oestrogen therapy at the age of 12 allows puberty evolution, without interfering with final height.[24] Before starting therapy, FSH levels must be determined in order to assess ovarian reserve and a pelvic ultrasound must be performed in order to determine uterine and ovarian dimensions.

In this study, puberty was induced with oral oestrogen in 76.7% of the patients and in the remaining 23.3% with transdermal oestrogen. The appropriate route of administration of oestrogen has not been determined in TS. The preferred pubertal induction protocol in patients without spontaneous puberty is a low-dose step-up transdermal oestrogen regimen (preferably of natural origin, as estradiol valerate), during about 2-3 years, until feminization occurs.[4,14] Based on few comparative data[25], transdermal oestrogen has the theoretical advantage of avoiding a first passage hepatic effect, thereby reducing the risk of hypertriglyceridemia and hypertension,

Progesterone introduction occurred, on average, at a chronological age of 17.2 ± 1.8 years and a mean bone age of 13.5 ± 1.1 years (about 1.1 years after the beginning of oestrogen). It´s use is recommended within a cyclic oestrogen regimen, when a uterine bleeding occurs or following 24 months of oestrogen therapy, in order to prevent endometrial hyperplasia.

A favorable reproductive organ development (uterus and ovaries) has been observed after pubertal induction (group 2), assessed by ultrasound. The initial uterine assessment showed a normal size in 20.4% and the presence of atrophy in the remaining 79.6% of the patients. At final ultrasound assessment, the prevalence of a normal uterus increased to 60.0%. In the same group, ovarian initial assessment showed a 92.6% percentage of atrophy. At final assessment, normal ovaries increased to 27.3% and atrophic ovaries were reduced to 72.7%. Therefore, in pubertal induction group, there was a significant difference between the initial and final ultrasound assessment as regards uterine ($p < 0.001$) and ovarian dimensions ($p = 0.004$).

In this group of 79 patients, two pregnancies occurred, both in patients with TS and induced puberty (group 2). One may have been spontaneous and non-evolutive in a patient with a 45,X karyotype. There have been descriptions of chromosomal abnormalities in the offspring and an increased incidence of spontaneous pregnancies in patients with TS. Furthermore, patients with structural abnormalities on one of the X chromosomes have a risk of transmitting this abnormality to their daughters.[26]

The other pregnancy resulted from an *in vitro* fertilization with oocyte donation in a patient with a mosaicism (45,X/46,XY), resulting in a twin pregnancy, apparently without any maternal or foetal complications. *In vitro* fertilization with oocyte donation and oocyte or ovarian tissue cryopreservation are the best possible assisted reproductive techniques in these patients, the former being the most commonly used.[27] Oocyte donation has been used amongst women with TS since treatment became available in the mid-eighties.[28] In the beginning, pregnancy results were not as good as those obtained in other patients with ovarian insufficiency,[29,30] although more recent researches have demonstrated similar pregnancy rates to those obtained in other women with ovarian failure.[31-35] Also, the abortion rate has been reduced, probably due to a more adequate hormone replacement therapy, with better uterine preparation. Pregnancy rates by embryo transfer have varied between 30 and 60%.[29-35]

Hypertension is a problem in oocyte donation pregnancies, affecting up to 30% of the patients in general[36,37] It also affects patients with TS who have other risk factors beyond hypertension. Aorta dissection is a major complication, occurring in approximately 2% of these patients each year.[38] Pregnancy may increase the risk of dissection in patients that already present with aortic dilatation. Therefore, before a planned pregnancy, MRI imaging is most definitely indicated to exclude this diagnosis. Considering these two risk factors and a likely increased risk of impaired glucose tolerance in these patients, the additional risk of twin pregnancies is unwelcome. As such single embryo transfer is required.

Adequate hormone replacement therapy, is recommendable for optimization of the uterus for pregnancy, preferably 4 to 6 months before embryo transfer.[33,39]

Cryopreservation of cortical ovarian tissue is largely

applied in young women before chemo or radiotherapy, with satisfactory pregnancies upon tissue thawing and subsequent transplant.[40-42] Technically, small fragments of ovarian tissue are obtained by laparoscopic biopsy, avoiding damage to the remaining tissue in order to reduce the chance of a future spontaneous pregnancy.

CONCLUSIONS

In the present study, most patients with a Turner syndrome did not present spontaneous puberty (74.7% *vs*. 25.3%).

Comparing group 1 (with spontaneous puberty) with group 2 (without spontaneous puberty), we observe statistical significant differences in karyotype distribution, in FSH levels and in uterine and ovarian size.

ACKNOLEDGEMENTS

To Margarida Marques, from the *Serviço de Tecnologias e Sistemas de Informação* of *Centro Hospitalar e Universitário de Coimbra*, for the biostatistics data processing.

CONFLICTS OF INTEREST

The authors declare the absence of any conflict of interests in writing this manuscript.

FINANCIAL SOURCES

The authors declare the absence of any financial sources. The present work was previously presented as an oral communication at the *XIV Congresso Português de Endocrinologia* and the 64th SPEDM Annual Meeting, which took place in Porto at the *Centro de Congressos* of the *Hotel Porto Palácio*, from the 24th to the 27th January 2013.

REFERENCES

1. Collett-Solberg P, Gallicchio C, Coelho S, Siqueira R, Alves S, Guimarães M. Endocrine diseases, perspectives and care in Turner syndrome. Arq Bras Endocrinol Metab. 2011;55:550-8.
2. Gravholt C. Clinical practice in Turner syndrome. Nat Clin Pract Endocrinol Metab. 2005;1:41-52.
3. Hook E, Warburton D. The distribution of chromosomal genotypes associated with Turner's syndrome: livebirth prevalence rates and evidence for diminished fetal mortality and severity in genotypes associated with structural X abnormalities or mosaicism. Hum Genet. 1983;64:24–7.
4. Davenport M. Approach to the patient with Turner syndrome. J Clin Endocrinol Metab. 2010;95:1487-95.
5. Saenger P, Wikland K, Conway G, Davenport M, Gravholt C, Hintz R, et al. Recommendations for the diagnosis and management of Turner syndrome. J Clin Endocrinol Metab. 2001;86:3061–9.
6. Saenger P. Clinical manifestations and diagnosis of Turner syndrome (gonadal dysgenesis). Uptodate [Consultado em 2012 Nov 16]. Disponível em: http://www.uptodate.com.
7. Weiss L. Additional evidence of gradual loss of germ cells in the pathogenesis of streak ovaries, J Med Genet.1971;8:540–4.
8. Singh R, Carr D. The anatomy and histology of XO human embryos and fetuses. Anat Rec. 1966;155:369–75.
9. Verlinde F. Foetal ovarian follicular reserve in Turner syndrome. In: P. Saenger, A.M. Pasquino, editors. Optimizing Health Care for Turner Patients in the 2001st Century. International Congress series no. 1212. Amsterdam: Elsevier; 2000. p.323.
10. Pasquino AM, Passeri F, Pucarelli I, Segni M, Municchi G. Italian Study Group for Turner's Syndrome. Spontaneous pubertal development in Turner's syndrome. J Clin Endocrinol Metab. 1997;82:1810–3.
11. Tarani L, Lampariello S, Raguso G, Colloridi F, Pucarelli I, Pasquino AM, et al. Pregnancy in patients with Turner's syndrome: six new cases and review of literature. Gynecol Endocrinol. 1998;12:83–7.
12. Hovatta O. Pregnancies in women with Turner's syndrome. Ann Med. 1999;31:106–10.
13. Bondy C. Turner syndrome 2008. Horm Res. 2009; 71:S52-6.
14. Alves S, Gallicchio C, Guimarães M, Santos M. Gonadotropin levels in Turner's syndrome: correlation with breast development and hormone replacement therapy. Gynecol Endocrinol. 2003;17:295-301.
15. Sybert V. Turner's syndrome. In: Cassidy SB, Allanson JE, editors. Management of genetic syndromes. 2nd ed. Wilmington: Wiley-Liss; 2005. p.589-605.
16. Mazzanti L, Nizzoli G, Tassinari D, Bergamaschi R, Magnani C, Chiumello G, et al. Spontaneous growth and pubertal development in Turner's syndrome with different karyotypes. Acta Paediatr. 1994;83:299-304.
17. Aso K, Koto S, Higuchi A, Ariyasu D, Izawa M, Igaki J, et al. Serum FSH level below 10 mIU/mL at twelve years old is an index of spontaneous and cyclical menstruation in Turner syndrome. Endocr J. 2010;57:909-13.
18. Gilboa Y, Rosenberg T. Typical Turner's syndrome with 45 XO karyotype and normal menstruation. Cytogenic and histological findings. Helv Paediatr Acta. 1975;30:281-8.
19. Jacquemun Y, Dumon J, Buytaert P. Ovarian function in the non-mosaic Turner sundrome; a case report. Eur J Obstet Gynecol Reprod Biol. 1989;30:87-191.
20. Cools M, Rooman R, Wauters J, Jacqemyn Y, Du Caju M. A nonmosaic 45,X karyotype in a mother with Turner's syndrome and in her daughter. Fertil Steril. 2004;82:923-5.
21. Styne D, Grumbach M. 45,X Turner's Syndrome. In: Kronenberg HM, Melmed S, Polonsky KS, Larsen PR, editors. Williams Textbook of endocrinology. 11th ed. Philadelphia: Saunders; 2008. p.1055-8.
22. Mortensen K, Cleeman L, Hjerrild B, Nexo E, Loc H, Jeppesen EM, et al. Increased prevalence of autoimmunity in Turner syndrome-- influence of age. Clin Exp Immunol. 2009;156:205-10.
23. Borgström B, Hreinsson J, Rasmussen C, Sheikhi M, Fried G, Keros V, et al. Fertility preservation in girls with turner syndrome: prognostic signs of the presence of ovarian follicles. J Clin Endocrinol Metab. 2009;94:74–80.
24. Cleemann L, Hjerrild B, Lauridsen A, Heickendorff L, Christiansen J, Mosekilde L, et al. Long-term hormone replacement therapy preserves bone mineral density in Turner syndrome. Eur J Endocrinol. 2009;161:251-7.
25. Werther G. Australasian Paediatric Endocrine Group. Turner Syndrome Management Guidelines. [consultado em 2012 Nov 09]. Disponível em: http://www.apeg.org.au/portals/0/documents/turner_posstate.pdf
26. Verschraegen-Spae MR, Depypere H, Speleman F, Dhondt M, De Paepe A. A familial Turner syndrome. Clin Genet. 1992;41:218–20.
27. Kavoussi S, Fisseha S, Smith Y, Smith G, Christman G, Gago L. Oocyte cryopreservation in a woman with mosaic Turner syndrome: a case report. J Reprod Med. 2008;53:223-2.
28. Gutiérrez Gutierrez AM, Grimalt L, Remohí J, Pellicer A. Twin pregnancy after oocyte donation in a woman with Turner syndrome. Ginecol Obstet Mex. 1994;62:82–4.
29. Rogers PA, Murphy CR, Leeton J, Hoise MJ, Beaton L. Turner's syndrome patients lack tight junctions between uterine epithelial cells. Hum Reprod. 1992;7:883–5.
30. Yaron Y, Ochshorn Y, Amit A, Yovel I, Kogosowki A, Lessing JB. Patients with Turner's syndrome may have an inherent endometrial abnormality affecting receptivity in oocyte donation. Fertil Steril. 1996;65:1249–52.
31. Press F, Shapiro HM, Cowell CA, Oliver GD. Outcome of ovum donation in Turner's syndrome patients. Fertil Steril. 1995;64:995–8.
32. Khastgir G, Abdalla H, Thomas A, Korea L, Latarche L, Studd J. Oocyte donation in Turner's syndrome: an analysis of the factors affecting the outcome. Hum Reprod. 1997;12:279–85.
33. Foudila T, Soderstrom-Anttila V, Hovatta O. Turner's syndrome and pregnancies after oocyte donation. Hum Reprod. 1999;14:532–5.
34. Delbaere A, Englert Y. Turner's syndrome and oocyte donation. Gynecol Obstet Fertil. 2002;30:970–8.
35. Bodri D, Vernaeve V, Figueras F, Vidal R, Guillén JJ, Coll O. Oocyte donation in patients with Turner's syndrome: a successful technique but with an accompanying high risk of hypertensive disorders during pregnancy. Hum Reprod. 2006;21:829–32.

36. Söderström-Anttila V, Tiitinen A, Foudila T, Hovatta O. Obstetric and perinatal outcome after oocyte donation: comparison with in-vitro fertilization pregnancies. Hum Reprod. 1998;13:483–90.
37. Wiggins D, Main E. Outcomes of pregnancies achieved by donor egg in vitro fertilization — a comparison with standard in vitro fertilization pregnancies. Am J Obstet Gynecol. 2005;192:2002–6.
38. Karnis MF, Zimon AE, Lalwani SI, Timmreck LS, Klipstein S, Reindollar RH. Risk of death in pregnancy achieved through oocyte donation in patients with Turner syndrome: a national survey. Fertil Steril. 2003;80:498–501.
39. Hovatta O, Hreinsson JG, Fridström M, Borgström B. Fertility and pregnancy aspects in Turner syndrome. International Congress Series. 2006;1298:185–9.
40. Donnez J, Dolmans MM, Demylle D, Jadoul P, Pirard C, Squifflet J, et al. Livebirth after orthotopic transplantation of cryopreserved ovarian tissue. Lancet. 2004;364:1405–10.
41. Meirow D, Levron J, Eldar-Geva T, Hardan I, Fridman E, Zalel Y, et al. Pregnancy after transplantation of cryopreserved ovarian tissue in a patient with ovarian failure after chemotherapy. N Engl J Med. 2005;353:318–21.
42. Hovatta O. Methods for cryopreservation of human ovarian tissue. Reprod Biomed Online. 2005;10:729–34.

Márcia ALVES, Margarida BASTOS, Teresa ALMEIDA SANTOS, Francisco CARRILHO

Gonadal Function in Turner Syndrome

Acta Med Port 2013:26:655-663
Publicado pela **Acta Médica Portuguesa**, a Revista Científica da Ordem dos Médicos

Av. Almirante Gago Coutinho, 151
1749-084 Lisboa, Portugal.
Tel: +351 218 428 215
E-mail: submissao@actamedicaportuguesa.com
www.actamedicaportuguesa.com
ISSN:0870-399X | e-ISSN: 1646-0758