Original Article

The Use of Fluorescence *In situ* Hybridisation in the Diagnosis of Hidden Mosaicism in Egyptian Patients with Turner Syndrome

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ABSTRACT

Background: Turner syndrome (TS) is the most common chromosomal abnormality in females. The diagnosis of TS is based on karyotyping of 30 blood lymphocytes. This technique does not rule out tissue mosaicism or low-grade mosaicism in the blood. Because of the associated risk of gonadoblastoma, mosaicism is especially important in case this involves a Y chromosome. **Aims:** This study was set to determine the value of additional genetic studies such as fluorescent *in situ* hybridisation and the inclusion of buccal cells in search for mosaicism in TS patients. **Settings and Design:** This cross-sectional, descriptive study was performed in Human Genetics Department, Medical Research Institute, Alexandria University. **Materials and Methods:** Fluorescence *in situ* hybridisation technique was applied to lymphocyte cultures as well as buccal smears using centromeric probes for X and Y chromosomes. Genotype phenotype correlation was also evaluated. **Statistical Analysis Used:** Descriptive study where categorical variables were described using number and percentage and continuous variables were described using mean and standard deviation. **Results:** Fluorescence *in situ* hybridisation technique study detected hidden mosaicism in 60% of studied patients; 20% of patients had a cell line containing Y material, while 40% had variable degrees of X, XX mosaicism, and in the remaining 40% no second cell line was detected. Fluorescence *in situ* hybridisation study helped identify the origin of the marker to be Y in all patients. The introduction of an additional cell line helped in identifying mosaicism in patients with monosomy X. Virilisation signs were only observed among TS patients with Y cell line mosaicism. The clinical manifestations were more severe in patients with monosomy X than other mosaic cases. **Conclusions:** Molecular cytogenetic investigation for all suspected cases of TS should be considered for appropriate treatment plan and genetic counselling.

KEYWORDS: *Fluorescent in situ hybridisation, mosaicism, turner syndrome*

INTRODUCTION

*T*urner syndrome (TS) is one of the most common cytogenetic abnormalities (1 in 2000 among live-born females). It is compatible with postnatal life, even though a great majority of conceptuses with this syndrome are spontaneously aborted.[1]

TS is caused by partial or complete X chromosome monosomy. Besides short stature and gonadal dysgenesis, TS is associated with a wide range of abnormalities affecting nearly every organ system. The phenotype is highly variable which could be explained by differences in karyotype, as well as the presence of tissue mosaicism.[2-5]

The concept of tissue mosaicism was established by the assumption that for a foetus to survive to term it

Access this article online

Quick Response Code:

Website:
www.jhrsonline.org

DOI:
10.4103/jhrs.jhrs_128_23

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is necessary to have a second sex chromosome. This implies that most, if not all live born girls with apparent 45, X, have additional cell lines in other tissues. This assumption is strengthened by the fact that complete 45, X is more common in foetuses who do not survive to term than in live born girls with TS.[6-8]

The karyotypic anomaly in 40%–60% of TS patients is monosomy (45, X), while the remainder has mosaicism with another cell line containing a normal or abnormal sex chromosome, although a small subset of patients have structurally abnormal X or Y chromosomes. Approximately 20% of mosaic TS have a sex marker chromosome[9] and 6% of these cases have a second cell line with a structurally abnormal Y chromosome.[10,11] Turner patients with a Y chromosome have a 30% risk of developing gonadoblastoma.[10,12] It has been suggested that a locus predisposing to gonadoblastoma development is located in the pericentromeric region of Yp.[13]

The diagnosis of TS is generally based on the cytogenetic analysis of 20–30 cultured blood lymphocytes, allowing only the identification of 10% of chromosomal mosaicism.[10,14,15] Standard karyotyping of merely blood lymphocytes in TS patients is insufficient in many cases,[10,11] due to inability to rule out tissue mosaicism. In addition, low-grade mosaicism (<10%) in blood lymphocytes can easily be missed during karyotyping, therefore additional techniques[6,16] such as polymerase chain reaction and fluorescent *in situ* hybridisation (FISH) have been advocated.[2,17] FISH with X-and Y-specific probes is used as a rapid and effective technique to detect sex chromosome mosaicism, as it can be performed on non-dividing cells (interphase), enabling the scoring of a larger cell number. In addition, it can be performed on blood or tissue samples, for example, buccal cells.[6,10,18]

The aim of the present study was to determine the value of additional genetic studies by FISH and the inclusion of a second tissue (buccal cells) in search for mosaicism in TS patients with apparent complete 45, X karyotype, as well as identifying the source of marker chromosomes in TS patients with 46,X, +mar on standard lymphocyte karyotyping. Genotype phenotype correlation was also evaluated.

Patients and Methods

The present study was carried out on 20 TS patients, diagnosed by karyotyping, recruited from Human Genetics Department, Medical Research Institute, Alexandria University. The research was reviewed and approved by the Ethics Committee (IORG#: IORG0008812), Medical Research Institute,

Alexandria University, adhering to the declaration of Helsinki (2013). The sample size was decided arbitrarily due to disease rarity and insufficient number of subjects studied.

All participants/their guardians were asked to freely volunteer to the study and informed written consents were gathered before their inclusion in the study, according to the Ethical Guidelines of the Medical Research Institute, Alexandria University.

All patients were subjected to detailed genetic and clinical history taking, including clinical genetic examination, abdominal and pelvic ultrasonography, echocardiography and hormonal assay (follicle-stimulating hormone [FSH], luteinising hormone [LH], T3 and T4, thyroid-stimulating hormone [TSH] and growth hormone [GH]) (basal and after stimulation levels).

The cytogenetic study was performed using the G-banding technique according to Seabright.[19] G-banded metaphase chromosomes were then examined at 550 band level according to the International System for Human Cytogenetic Nomenclature (ISCN 2020).[20]

Fluorescence *in situ* hybridisation technique was applied to lymphocyte cultures as well as freshly prepared buccal smears using Cytocell aquarius kit (REF: LPu XYc) for X chromosome centromere, Xp11.1-q11.1 (DXZ1) Green and Y chromosome centromere, Yp11.1-q11.1 (DYZ3) Orange. Probes and slide preparations as well as hybridisation and washing techniques were performed according to the manufacturer's protocols as follows:

FISH analysis was performed on cell samples from lymphocyte cultures and buccal smears for each patient. 10 µl of the probe was applied to the sample, co-denatured and hybridised using the Thermobrite Slide Processing System (Leica ThermoBrite System/USA). Posthybridisation wash was performed using 0.4 × SSC/0.3NP-40 kept in 72 ± 1°C and 2 × SSC/0.1%NP-40 kept at room temperature for 2 min each, finally, counterstain was applied. The slides were scanned under the fluorescent microscope (Olympus/BX53). Image capture was done using digital high-resolution camera (JENOPTIK: D-007739Jena) (Olympus, Japan) and the software Auto image analysis for FISH and karyotyping LUCIA.

For each patient, a total of 500 interphase and twenty metaphase nuclei from lymphocyte culture and a total of 200 buccal cells were analysed.[21,22] A classical TS female with 45, X karyotype will show only one green signal. Any degree of mosaicism will be counted and considered positive if they were ≥ 5% in blood and buccal cells.[8,22,23]

Statistical analysis

Descriptive study where categorical variables were described using number and percentage and continuous variables were described using mean and standard deviation (SD).

RESULTS

The age of the studied patients at the time of initial examination ranged from 12 days to 38 years (mean 12.15, SD ± 9.02). Five patients (25%) presented in infancy with dorsal pedal oedema and neck webbing, seven patients (35%) presented in childhood with short stature, seven patients (35%) presented over 14 years of age with short stature, as well as primary amenorrhea. One patient (5%) aged 38 years presented with primary infertility [Table 1].

All studied TS patients were isolated cases with negative family history and unremarkable pregnancy history. Five patients (25%) had low birth weight; otherwise, delivery history was unremarkable. Congenital heart disease (CHD) was detected in five patients (25%), renal pathology was found in five patients (25%) and recurrent ear infections were reported in two patients (10%).

The results of clinical examination of the studied patients are summarized in Table 2. Fifteen patients (75%) were below the 3rd percentile on age appropriate growth curves, while five patients (25%); namely, an 18 years old patient with mos45, X/46,X, +mar karyotype, and four patients with 45,X karyotype had normal height between the 10th and the 25th percentile on age appropriate curves. Except for five patients (25%) who had concomitant GH deficiency whose weight fell below the third percentile; weights of all studied subjects were within the normal range for their ages. The head circumference was within normal range for all cases.[24]

The heights of the studied TS patients aged 14 years or older, marking the end of the female growth spurt, were less than their target final heights, calculated from their corresponding mid-parental heights, in all studied patients (100%) [Table 3].

The most common clinical features encountered were low posterior hairline (100%), shield chest and widely spaced nipples (95%), short stature, neck webbing, short 4th and 5th metacarpals and low set and/or malformed ears (75%) and high-arched palate (70%). Cubitus valgus was observed in 55%, short neck ± webbing in 50%, hypoplastic nails in 45%, multiple facial nevi in 35%, brachymetatarsia in 30% [Figure 1] and dorsal pedal oedema in 25%. Less frequently observed features were micrognathia (15%), ptosis (10%), squint (5%) and Madelung deformity (5%).

Table 1: Distribution of the studied turner syndrome patients according to the age and major presenting features

Major presenting features	Age (years)	*n* (%)
Oedema of the dorsum of the feet and neck webbing	0–1	5 (25)
Short stature	>1–14	7 (35)
Primary amenorrhea and short stature	>14–19	7 (35)
Primary infertility	≥20	1 (5)

Table 2: The frequency of clinical and radiological features among the studied patients

Clinical features	Number of patients (%)
Low posterior hair line	20 (100)
Shield chest	19 (95)
Short stature	15 (75)
Neck webbing	15 (75)
Short fourth and fifth metacarpals	15 (75)
Large and/or malformed ears	15 (75)
High arched palate	14 (70)
Cubitus valgus	11 (55)
Short neck	10 (50)
Hypoplastic nails	9 (45)
Nevi	7 (35)
Pedal oedema	5 (25)
Brachymetatarsia	5 (25)
Micrognathia	3 (15)
Hirsutism	2 (10)
Ptosis	2 (10)
Squint	1 (5)
Hearing loss	1 (5)
Madelung deformity	1 (5)
Hypoplastic (absent) 4th and 5th toes	1 (5)
Receding and thinning of frontal hairline	1 (5)
Radiological investigation	
I - Ultrasonography pelvis	
Small sized ovaries and uterus	18 (90)
II - MRI pelvis	
Complete mullerian agenesis	2 (10)
III - Echo	
Cardiac anomalies	5 (25)
IV - Ultrasound abdomen renal view	
Renal anomalies	4 (20)

MRI=Magnetic resonance imaging

The study group was also examined for the presence of virilisation signs, which were encountered in three patients (15%) in the form of hirsutism in two patients (10%) and frontal hair thinning in one patient (5%).

Some unusual features were encountered in the studied subjects; one patient (5%) had an extra tooth behind

Patient number	Age (years)	Measured height (cm)	Mid-parental height expected range (cm)	Target final height (cm)
1	18	153	165.5–173.5	165
4	19	141	143–160	151.5
6	19	144	145–162	153.5
7	15	144	157.5–174.5	166
8	38	142	NA	NA
10	14	135	160–177	168.5
12	19	143	152.5–169.5	161
13	14	122	145–162	153.5
16	19	148	NA	NA
19	14	151	152.5–169.5	161

Table 3: Heights of the studied turner syndrome patients (≥14 years) in correlation with the mid-parental height

*Parents who couldn't be assessed at the time of the study because they were unavailable

central incisors, another patient (5%) had severely hypoplastic 5th toenail and absent 4th and 5th toes bilaterally.

Pelvic sonography revealed small or hypoplastic uterus and ovaries in 90% of cases and two patients (10%) had non-visualised or absent uterus. On abdominal sonography, four cases (20%) had renal anomalies in the form of horseshoe kidney in two cases, bilateral malrotated kidneys in one case and bilaterally dilated renal pelvis in another. Two patients (10%) had complete Mullerian agenesis and non-visualised ovaries on magnetic resonance imaging pelvis. Echocardiography was normal in 15 patients (75%), while the other five (25%) cases had CHD in the form of atrial septal defect in two cases, atrioventricular concordance in one case, one patient showed aortic coarctation and another case showed post ductal aortic coarctation as well as patent ductus arteriosus.

FSH and LH levels were requested in all studied patients, assessed in 15 patients yielding an increased FSH level ranging from 44.5 to 179 mIU/ml, while the other five patients were too young for assessment. Similarly, LH level was above normal, ranging from 8.16 to 45.7 mIU/ml.[25]

Thyroid hormonal assay (T3, T4 and TSH) yielded normal results in all studied cases, GH level was assessed in 15 patients complaining of short stature and yielded low basal and after stimulation levels in five subjects 33.3% (5/15).

45,X karyotype was detected in 17 subjects (85%), mos45, X/46,X + mar in 2 cases (10%), while one patient (5%) had 46,X, +mar karyotype [Table 4].

FISH study was conducted on the study group using two tissues: Blood culture and buccal tissue. It revealed that four patients (20%) had a cell line containing Y material, 8 (40%) had variable degrees of X/XX mosaicism

Table 4: Distribution of cytogenetic abnormalities detected by karyotyping among the studied patients

Karyotype	Number of patients (%)
45,X	17 (85)
mos45, X/46,X,+mar	2 (10)
46,X,+mar	1 (5)

Figure 1: Patients 3, 4 and 10: Brachymetatarsia in three different Turner syndrome patients aged 19 years (a), 14 years (b) and 9 years (c) respectively

and in the remaining 8 (40%) no other cell lines were detected [Figures 2-5].

Regarding FISH study on blood culture, the origin of the marker chromosome in the two cases with mos 45, X/46,X, +mar [Figure 6] and the case with 46,X, +mar was found to be Y chromosome (100%). These two cases were found to have an additional cell line containing XYY by FISH. In the 17 cases with 45,X karyotype, 8 had an additional cell line; an XX in 6 cases (35.3%) and XY in one (5.9%), whereas, one case (5.9%) had two additional cell lines (XX, XXX) in addition to the
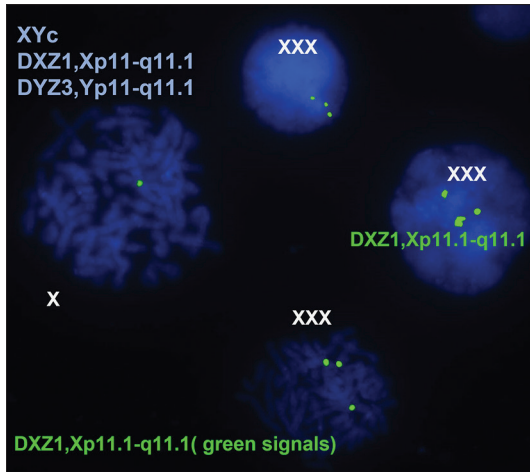
Figure 2: Blood fluorescent *in situ* hybridisation of a Turner syndrome patient with 45,X karyotype showing 2 interphases with (3 green) signals XXX and 2 metaphases one with (1 green) X signal and the other with (3 green) XXX
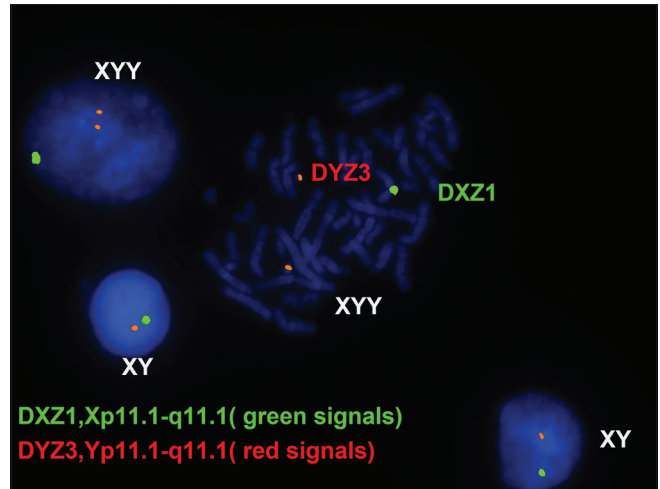

Figure 3: Blood fluorescent *in situ* hybridisation of a Turner syndrome patient with mos 45,X/46, X,+mar showing a metaphase with (2 red, 1 green) signals XYY, 3 interphases one with (2 red, 1 green) XYY and 2 with (1 red, 1 green) signals XY
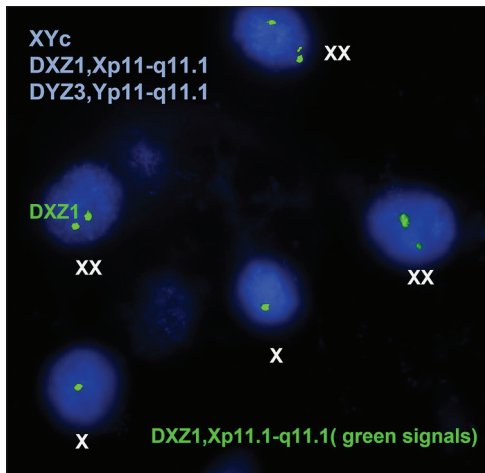

Figure 4: Buccal fluorescent *in situ* hybridisation of a Turner syndrome patient with 45,X karyotype showing 5 buccal cells 3 cells with (2 green) signals XX, and 2 cells with (1 green) signal X
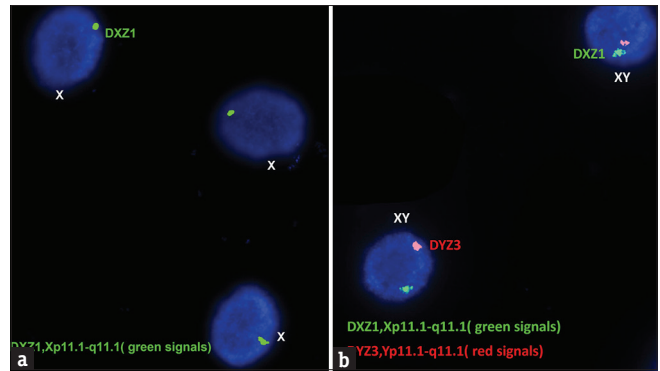

Figure 5: Buccal fluorescent *in situ* hybridization of a Turner syndrome patient with 45,X karyotype showing (a). 3 buccal cells with (1 green) signal X (b). 2 buccal cells with (1 red, 1 green) XY

45,X cell line. For the remaining 9 cases (52.9%), only one cell line with monosomy X was detected.

Regarding FISH study on buccal tissue, the origin of the marker chromosome in the two patients with mos45, X/46, X, +mar and one patient with 46,X, +mar was confirmed to be Y chromosome in all the three patients (100%). The three patients were confirmed to have XY cell line, while XYY cell line was detected in only one patient with mos45, X/46,X, +mar karyotype. In the 17 patients with 45, X karyotype, 9 had an additional cell line: XX in 8 (47.05%) and XY in one patient (5.9%). Regarding the remaining eight patients (47.05%), only one cell line with monosomy X was detected.

Different results were encountered between the two tissues used for FISH: blood culture and buccal tissue [Tables 5 and 6].

One case with 45, X karyotype (patient 4) had a second cell line containing XX in buccal FISH but, not in blood FISH. In another 45,X karyotype case (patient 6), the percentages of the two detected cell lines were different between blood and buccal tissue; 85.5% for X cell line and 14.5% for XX cell line in blood and 38% for X cell line and 62% for XX cell line in buccal tissue. In a third 45, X karyotype case (patient 9), three cell lines were detected in blood X, XX and XXX while only two cell lines were detected in buccal tissue X and XX.

The difference in results between blood and buccal FISH was also seen in a 46, +mar karyotype case (patient 10), in whom three cell lines were detected in blood; X, XY, and XYY, but only two cell lines; X, XY were detected in buccal tissue.

Otherwise, similar cell lines were detected by blood and buccal FISH among patients but with different percentages of mosaicism.
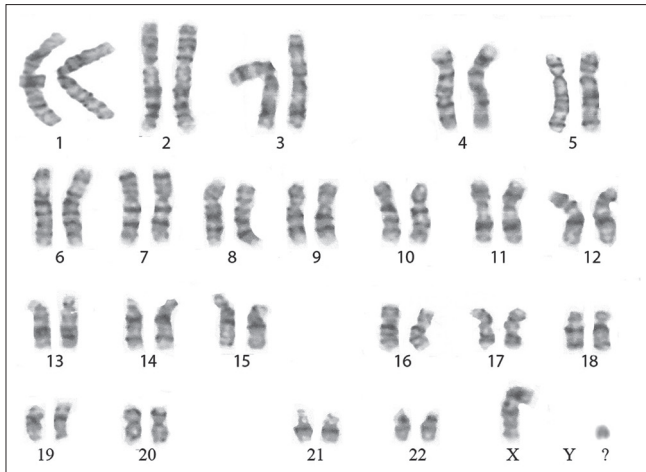
Figure 6: Karyotype of patient one showing 46,X,+mar

All studied TS patients were found to have a cell line containing monosomy X with/or without mosaicism with another cell line, whether containing X chromosome or Y chromosome. FISH results were correlated with clinical features and presented in Tables 6, 7 and Figure 7.

The mean age at the diagnosis was 12.15 years for all patients, 6.79 years for patients with monosomy X, 16.46 years for patients with mosaic X cell lines and 14.25 years for patients with mosaic Y cell lines.

Short stature was a consistent feature in all adult cases whether with monosomy X cell line or with mosaic X and Y cell lines, except for one case with mosaic Y cell line who showed normal height.

Neck webbing frequency was the same for patients with monosomy X cell line and those with mosaic X cell lines, but it was reported less frequently in patients with mosaic Y cell lines.

Regarding facial dysmorphic features, low set and/or malformed ears was the most commonly obseved feature in the studied group, occurring with the highest frequency in mosaic X subjects (87.5%), followed by monosomy X (75%) and least in mosaic Y cases (50%).

The frequency of wide carrying angle (cubitus valgus) was the highest in patients with mosaic X cell lines (62.5%), occurring in 50% of cases with 45, X and 50% of cases with mosaic Y cell lines. Short 4th and 5th metacarpals were observed with similar prevelance (87.5%) in subjects with 45, X as well as subjects with mosaic X cell lines, while encountered in only 25% of patients with mosaic Y cell lines. A more severe skeletal anomaly was recorded in one patient with one cell line-containing monosomy X in the form of fibular ray limb defect with absent 4th and 5th toes bilaterally.

Lymphoedema of the dorsum of the feet was present in 50% of patients with monosomy X cell line, and in 12% of cases with mosaic X cell line, while it was not encountered in subjects with mosaic Y cell lines.

Virilisation signs such as facial hirsutism, broad forehead, frontal hair thinning and bushy eye brows were reported only in patients with mosaic Y cell lines.

Pelvic ultrasonography showed that 90% of studied TS patients had hypoplastic or small uterus ± ovaries, while a more severe phenotype in the form of complete mullerian agenesis was found in two patients (10%); one with monosomy X and the other with mosaic X cell line.

CHD was reported only in cases with monosmy X cell line, and renal malformations were found with the highest frequency in monosomy X patients (75%), followed by those with mosaic X cell lines (25%), while none of the subjects with mosaic Y cell lines had congenital cardiac or renal malformations.

Follow-up of TS cases along the 2 years course of the study revealed disappearance of dorsal feet oedema in all involved patients over the course of 6–12 months, one case (patient 2) developed conductive hearing loss at the age of 5 years, while another (patient 1), in which Y chromosome was detected, had undergone gonadectomy.

DISCUSSION

TS is a common chromosomal disorder, combining many characteristic physical features with complete or partial absence of the second sex chromosome, with or without cell line mosaicism.[26] The range of morbidities associated with TS can have a profound effect on the quality of life, and there is a clear need for an integrated multidisciplinary approach to treatment.[27,28]

Marker chromosomes can be detected in about 20% of mosaic TS which could be derived from the X or Y chromosome, and in 6% of cases the marker is derived from a structurally abnormal Y chromosome.[29] TS patients with a Y chromosome have 30% risk of developing gonadoblastoma.[12]

Blood lymphocyte karyotype is the gold-standard method for the diagnosis of TS. However, some studies suggest that, in some cases, it could miss low-level mosaicism or the presence of cells containing a Y chromosome.[30] In addition, the frequency of abnormal cells can vary from one tissue to another.[31] In such cases, an additional diagnostic test for detection of mosaicism should be performed[32] which can be achieved by DNA hybridisation or FISH using X and Y centromeric probes.[33]

Table 5: The distribution of karyotype, blood and buccal fluorescent *in situ* hybridisation among the studied turner syndrome patients

	Karyotype blood	FISH blood*	FISH buccal**
Patients with mosaic Y cell lines	Mos45,X[42%]/ 46, X,+mar[58%]	Interphase X[20%]/XY[80%]	X[26%]/XY[74%]
		Metaphase X[30%]/XY[70%]	
	45,X[50%]/ 46, X, +mar[50%]	Interphase X[22%]/XY [53%]/XYY[25%]	X[49%]/XY[30%]/ XYY[21%]
		Metaphase X[25%]/XY[75%]	
	46,X,+mar	Interphase X[15%]/XY[82%]/XYY[3%]	X[40%]/XY[60%]
		Metaphase X[15%]/XY[81%]/XYY[4%]	
	45,X	Interphase X[91.6%]/XY[8.4%]	X[85.5%]/XY[14.5%]
		Metaphase X[85%]/XY[15%]	
Patients with mosaic X cell lines	45,X	Interphase X[100%]	X[81.5%]/XX[18.5%]
		Metaphase X[100%]	
	45,X	Interphase X[85.6%]/XX[14.4%]	X[38%]/XX[62%]
		Metaphase X[65%]/XX[35%]	
	45,X	Interphase X[81.2%]/XX[18.8%]	X[88.5%]/XX[11.5%]
		Metaphase X[85%]/XX[15%]	
	45,X	Interphase X[93.8%]/XX[6.2%]	X[90%]/XX[10%]
		Metaphase X[85%]/XX[15%]	
	45,X	Interphase X[87%]/XX[11%]/XXX[2%]	X[92%]/XX[8%]
		Metaphase X[70%]/XX[15%]/XXX[15%]	
	45,X	Interphase X[95%]/XX[5%]	X[94.5%]/XX[5.5%]
		Metaphase X[95%]/XX[5%]	
	45,X	Interphase X[93.6%]/XX[6.4%]	X[94%]/XX[6%]
		Metaphase X[90%]/XX[10%]	
	45,X	Interphase X[82%]/XX[18%]	X[79%]/XX[21%]
		Metaphase X[100%]	
Patients with one cell line monosomy X	45,X	Interphase X[100%]	X[100%]
		Metaphase X[100%]	
	45,X	Interphase X[100%]	X[100%]
		Metaphase X[100%]	
	45,X	Interphase X[100%]	X[100%]
		Metaphase X[100%]	
	45,X	Interphase X[100%]	X[100%]
		Metaphase X[100%]	
	45,X	Interphase X[100%]	X[100%]
		Metaphase X[100%]	
	45,X	Interphase X[100%]	X[100%]
		Metaphase X[100%]	
	45,X	Interphase X[100%]	X[100%]
		Metaphase X[100%]	
	45,X	Interphase X[100%]	X[100%]
		Metaphase X[100%]	

*Each patient had 500 interphase cells examined, **Each patient had 200 buccal cells examined. FISH=Fluorescent *in situ* hybridisation

The age of the patients at time of 1st examination, ranged from 12 days to 38 years with mean age of (12.15 ± 9.02 SD) years which is in accordance with published data.[34,35] Twenty-five percent of the studied patients were diagnosed in infancy with dorsal pedal oedema, neck webbing ± congenital anomalies, 35% were diagnosed in childhood with short stature, while the remaining 40% were diagnosed in adulthood with short stature, primary amenorrhea and/or infertility, which goes in agreement with other studies.[36]

Seventy-five percentage of the studied patients had short stature; the remaining 25% were within normal height, which goes in concordance with published data as TS patients may not be short at birth but short

Table 6: Clinical phenotypes and fluorescent in-situ hybridisation results among the studied turner syndrome patients

Patient	Age	Short stature	Webbed neck	Congenital lymphoedema	Wide carrying angle	Underdeveloped breast according to age	Scanty pubic and axillary hair	Virilization signs	Small size uterus ±ovaries on us	Cardiac anomalies	Renal anomalies	FISH results* Blood	FISH results* Buccal
1	18 years	X	X	X	X	X	X	√	√	X	X	X[20%]/XY[80%]	X[26%]/XY[74]
2	3 months	√	√	√	NA	NA	NA	X	√	√	X	X[100%]	X[100%]
3	12 days	√	√	√	√	NA	NA	X	√	√	√	X[100%]	X[100%]
4	19 years	√	√	X	√	√	X	X	√	X	X	X[100%]	X[81.5%]/XX[18.5%]
5	10 years	√	√	X	√	NA	X	X	√	√	X	X[100%]	X[100%]
6	19 years	√	√	X	√	√	X	X	√	X	X	X[85.6%]/XX[14.4%]	XX[62%]/X[38%]
7	15 years	√	√	X	√	√	√	X	Mullerian agenesis	X	X	X[81.2%]/XX[18.8%]	X[88.5%]/XX[11.5%]
8	38 years	√	X	X	X	√	X	X	√	X	X	X[93.8%]/XX[6.2%]	X[90%]/XX[10%]
9	8 years	√	√	X	√	X	√	X	√	X	X	X[87%]/XX[11%]/XXX[2%]	X[92%]/XX[8%]
10	14 years	√	X	X	X	√	X	√	√	X	X	XY[82%]/X[15%]/XYY[3%]	XY[60%]/X[40%]
11	13 days	X	√	√	NA	NA	NA	X	√	√	√	X[100%]	X[100%]
12	19 years	√	X	X	X	√	√	X	√	X	X	X[100%]	X[100%]
13	14 years	√	√	X	√	√	√	X	√	X	X	X[91.6%]/XY[8.4%]	X[85.5%]/XY[14.5%]
14	11 years	√	X	X	√	√	X	√	√	X	X	XY[53%]/XYY[25%]/X[22%]	X[49%]/XY[30%]/XYY[21%]
15	13 years	√	√	X	X	√	√	X	√	X	X	X[95%]/XX[5%]	X[94.5%]/XX[5.5%]
16	19 years	√	√	X	√	√	√	X	√	X	√	X[93.6%]/XX[6.4%]	X[94%]/XX[6%]
17	1 years	X	√	√	NA	NA	NA	X	√	√	√	X[100%]	X[100%]
18	10 years	√	√	X	√	NA	√	X	√	X	X	X[100%]	X[100%]
19	14 years	X	√	X	√	X	X	X	Mullerian agenesis	X	X	X[100%]	X[100%]
20	8 months	X	√	√	NA	NA	NA	X	√	X	X	X[82%]/XX[18%]	X[79%]/XX[21%]

*Cell-lines are written based on frequency, the one with higher percentage first. FISH=Fluorescent in-situ hybridisation. NA=Not available

Table 7: Clinical correlation with fluorescent *in situ* hybridisation, results in the studied turner syndrome patients

Age at presentation	X (*n*=8)	mos X (*n*=8)	mos Y (*n*=4)
	0.0329-19 years (mean 6.79±6.98)	0.6667–38 years (mean 16.46±10.11)	11–18 years (mean 14.25±2.49)
Clinical signs			
Low posterior hair line (*n*=20)	8/8	8/8	4/4
Shield chest (*n*=19)	8/8	8/8	¾
Short stature (*n*=15)	5/8	7/8	¾
Neck webbing (*n*=15)	7/8	7/8	¼
Short metacarpals (*n*=15)	7/8	7/8	¼
Low set and/or malformed ears (*n*=15)	6/8	7/8	2/4
High-arched palate (*n*=14)	4/8	8/8	2/4
Wide carrying angle (*n*=11)	4/8	5/8	2/4
Hypoplastic nails (*n*=9)	5/8	3/8	¼
Primary amenorrhoea (*n*=8)	1/8	5/8	2/4
Multiple nevi (*n*=7)	3/8	3/8	¼
Short metatarsals (*n*=6)	3/8	2/8	¼
Lymphoedema of extremities (*n*=5)	4/8	1/8	0/4
Virilisation signs (*n*=3)	0/8	0/8	¾
Ptosis (*n*=2)	0/8	1/8	¼
Squint (*n*=1)	1/8	0/8	0/4
Limb reduction (absent 4th and 5th toes) (*n*=1)	1/8	0/8	0/4
Hearing loss (*n*=1)	1/8	0/8	0/4
Radiological investigations			
Hypoplastic uterus±ovaries (*n*=18)	7/8	7/8	4/4
Mullerian agenesis (*n*=2)	1/8	1/8	0/4

Figure 7: Patient 17: A 1-year-old Turner syndrome patient with 45,X karyotype and no mosaicism detected on either blood or buccal fluorescent *in situ* hybridization showing (a). Neck webbing (b). Widely spaced hypoplastic nipples, shield chest (c). Hypoplastic nails more severe at 5th finger (d and e). Severe hypoplastic absent 4th and 5th toes on both right, left feet respectively (f). A plain X-ray of the feet showing absent terminal phalanges in 4th and 5th toes

stature presents later with reduced growth velocity, often delaying the diagnosis of short stature.[37,38] In agreement, some studies reported that many mosaic TS patients were of normal height,[39] while others[40] found that only 31% of TS patients with Y mosaicism have short stature. The absence of short stature in mosaic TS patients and TS patients with Y chromosomal material could be explained by the theory that mosaic forms of TS do not lack as many copies of the SHOX gene.[41]

Mid parental height was found to have an effect on final height of TS patients in agreement with others.[42-44]

Craniofacial features were most commonly low posterior hairline, followed by neck webbing, low set and/or malformed ears, high-arched palate, multiple facial nevi and micrognathia. Similar results were published with the most common dysmorphic features being low posterior hairline, high-arched palate and multiple pigmented nevi,[45] while lower frequencies of neck webbing (22%, and 18%) were reported by others.[46,47] These discrepancies in results may be attributed to differences in sample size, age of diagnosis, ethnicity and GH therapy.

The skeletal manifestations we reported were shield chest and widely spaced nipples, short 4th and 5th metacarpals, cubitus valgus and Madelung deformity. Other studies[48] reported short 4th metacarpal and cubitus valgus to be the most commonly encountered skeletal manifestations. In contrast, others[45] reported lower frequencies of cubitus valgus (36%), and short 4th and 5th metacarpals (20%). The discrepancies in results between studies can be attributed to differences in sample size, age of diagnosis and the degree of mosaicism in the studied patients.

Brachymetatarsia was observed in 25% of cases. There were three case reports of TS with brachymetatarsia.[49-51] Congenital brachymetatarsia is a rare condition that arises

from premature closure of the metatarsal epiphyseal plate. Females are almost exclusively affected, and the fourth metatarsal is the most frequently involved.[49,52] Early closure of the distal epiphysis is likely related to SHOX deficiency, it is rarely symptomatic and is most often only found incidentally on foot X-ray.[41]

Blood lymphocyte FISH analysis in patients with 45, X revealed mosaicism in 47% of cases; X chromosome cell line was detected in 41.1%, while Y chromosome cell line was detected in 5.9%. Others[23] reported mosaicism with blood lymphocyte FISH on apparently non-mosaic 45, X patients in 37%, all had X-derived cell lines with no Y-derived cell lines. Other studies[53] reported 22.2% of studied TS patients with 45, X having Y-derived cell line mosaicism. Similar published data[54] detected Y chromosome mosaicism in 3.3% of studied TS patients with 45, X. The discrepancies of the results may be attributed to the differences in sample size, the number of scored cells and the variability in the selection criteria.

Buccal FISH analysis in 45, X TS patients revealed mosaicism with a second cell line in 52.9% of cases. X chromosome containing cell line was detected in 47%, while Y chromosome containing cell line was detected in 5.9%, which goes in accordance with similar studies,[55] who reported mosaicism in 45, X TS patients using buccal FISH analysis, with detection of both X and Y chromosome containing cell lines.

Mosaicism was detected in 60% of studied TS patients using FISH analysis on both blood culture and buccal tissue, revealing X chromosome containing cell line in 40% and Y chromosome containing cell line in 20%. These results go in agreement with other studies,[21,56]

Mosaicism in buccal cells was detected in 52.9%, whereas mosaicism in blood lymphocytes was detected in 47% of studied TS patients, in agreement with published sudies.[21] A difference between blood and buccal tissue as regards the number of mosaic cell lines was noted in the present study; which goes in agreement with similar studies.[57,58]

These differences between blood and buccal FISH analysis can be explained by the different embryonic origin of each tissue, buccal cells originate from ectoderm while mesoderm is the embryonic origin of blood. Studying different tissues in TS is important for the detection of cryptic mosaics as mosaicism may not be detected in peripheral blood, but may be significant in tissue samples of different embryonic origin.[6,21,59]

In the current study, Y chromosome was the origin of the marker detected in the three patients with 46,X, +mar cell line, and it was identified by blood lymphocyte and buccal FISH, in agreement with other reports,[53,60] who identified the origin of the marker present among their studied patients to be Y chromosome using FISH.

The mean age of the diagnosis among patients with 45, X was 6.79 years, while the age of diagnosis in patients with mosaic Y and mosaic X cell lines was 14.25 and 16.46 years respectively, in agreement with published data.[61,62] This is attributed to the characteristic phenotype features in patients with monosomy X, alerting physicians earlier.

Both webbed neck and short 4th and 5th metacarpals were found with similar frequencies in patients with monosomy X cell line and those with mosaic X cell lines (87.5%), but they were observed less frequently in subjects with mosaic Y cell lines (25%), which agrees with published reports.[63]

Cubitus valgus was reported more frequently among cases with mosaic X cell line (62.5%) than those with monosomy X cell line and mosaic Y cell line (50%). These results are in disagreement with others[40,63] who reported cubitus valgus more frequently among patients with monosomy X than those with mosaic cell lines. These discrepancies can be explained by the age of the subjects studied, the degree of mosaicism and the possibility of tissue mosaicism.

Dorsal feet lymphoedema was detected in 50% of cases with monosomy X cell line, 12% of subjects with mosaic X cell line, while it wasn't detected in patients with mosaic Y cell lines, which agreed with published data.[45,61] Lower frequencies of lymphoedema among studied TS patients were reported in 15% of monosomy X and in 5% of mosaic X cases.[48] This discrepancy may be attributed to differences in sample size and patients age, because initial peripheral lymphoedema in TS is temporary and often resolves spontaneously during the first few months of life.

Virilisation signs were present only in patients with mosaic Y cell lines. This is in agreement with comparable studies[64] detecting virilisation signs only in Y material positive TS patients.

In the current study one patient with monosomy X developed conductive hearing loss at the age of 5 years. This is in agreement with other studies[65] reporting more severe hearing loss in patients who lack a short arm of an X chromosome compared with those with mosaicism. CHD was observed only in patients with monosomy X cell line, while renal malformations were found with highest frequency in cases with monosomy X (75%), followed by those with mosaic X cell

lines (25%), while none of the subjects with mosaic Y cell lines had congenital cardiac or renal malformations. Others,[61] reported CHD in 50% of monosomy X and 22% of mosaic TS, and renal malformations in 13% of monosomy X and 14% of mosaic TS. Comaparable studies,[48] reported CHD in 24% of monosomy X and in 14% of mosaic X cell lines TS patients, and renal malformations in 30% of monosomy X and in 43% of mosaic X. These discrepancies may be attributed to the differences in sample size and age of studied patients.

The most severe phenotype was encountered in monosomy X patients when compared to TS cases with mosaic cell lines. Congenital malformations, lymphatic and skeletal phenotypes were present at higher frequencies in monosomy TS patients. This agreed with the studies that reported more severe clinical manifestations in TS with monosomy X rather than other forms of TS.[40,45,61,63,66]

CONCLUSIONS

Chromosomal investigation for all the suspected cases of TS should be considered to approach an appropriate treatment plan and genetic counselling. FISH technique could detect mosaicism in TS which was not detected by conventional cytogenetic studies. The application of FISH technique on two tissues of different embryonic origin enables more accurate detection of tissue mosaicism in TS patients.

Author's contributions

HO: Data acquisition, data analysis and drafting the work. SK: Design of the work; interpretation of data and revision. GE: Design of the work; interpretation of data and revision. All authors read and approved the final manuscript.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

Data availability statement

Available on request.

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