

## Comparison of classical cytogenetics versus interphase FISH in diagnosis of mosaic form of Turner syndrome

Seyed Mojtaba Mohaddes<sup>1</sup>, Shohreh Shargh<sup>2</sup>, Simin Tagavi<sup>3</sup>

*Department of Medical Genetics, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran.*

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### Abstract

**Background:** Mosaic form of turner syndrome that represented by two or more cell lines in an affected individual, often has limitation for detection with classical cytogenetic methods. The present study was carried out to compare the efficiency of interphase Fluorescence In Situ Hybridisation (FISH) and cytogenetic techniques in detection of mosaic form of turner syndrome

**Methods:** All candidate samples for turner syndrome were surveyed with both interphase FISH using DXZ1 as a chromosome X specific probe and the GTG- banding methods. The chi square test was used and a P-value of less than 0.05 was considered as being significant.

**Results:** A significant difference was observed between results obtained from the application of the two methods under study ( $P < 0.05$ ), indicating that the interphase FISH is favourably compares to conventional cytogenetics in detection of mosaic form of X chromosome aneuploidy, as an extended number of cells can be scored in a limited time.

**Conclusion:** The results indicate that using the two techniques in parallel allow accurate differentiation between mosaicism and homogenous aneuploidy of X chromosome, and thus both numerical and structural aberrations of the X will be analyzed.

**Keywords:** Turner syndrome, mosaicism, FISH, classical cytogenetics.

### Introduction

Mosaicism is defined as presence of two or more cell lines in an individual, derived from a single zygote. About 24% of patients affected by Turner syndrome (TS), are mosaics for normal and monosomic cell lines. Similar conditions are observed in other aneuploidies [1]. The presence of normal cell line in individuals with mosaic aneuploidies tends to moderate the clinical picture. However if the abnormal cell

line is limited to the gonad (gonadal mosaics, in the case of X chromosome aneuploidy), abnormal sexual development may occur [2].

Numerical and structural chromosome abnormalities are routinely diagnosed using standard cytogenetic techniques. However the detection of chromosomal mosaicism is often difficult due to long duration and limited number of available metaphase cells for commencing analysis. Interphase fluorescence in situ hybridisation (FISH) which employs hybridisa-

1. **Corresponding author**, Department of Medical Genetics, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran, Tel/Fax: 0098411-3364666, mohaddesmo@yahoo.com

2. PhD student of Medical Genetics. Email: shohreshshargh@gmail.com.

3. Associate professor of Obstetrics and Gynecology. Email: taghavi220@msn.com.

tion of specific labelled probes on target DNA in interphase cells, can overcome the limitations encountered in standard cytogenetic methods. The technique has valuable application in detection of chromosomal mosaicism since a minimum of 100 cells can be analysed in short period of time [3].

The detection efficiency of interphase FISH depends on the specificity and sensitivity of the probes which are used. Centromeric probes are the most suitable and widely used probes for aneuploidy detection by interphase FISH. These probes generally hybridise to highly repetitive DNA sequences clustered in the centromeric regions of human chromosomes, producing strong and intense fluorescent signals on their specific targets. A variety of highly repetitive DNA sequences including alphoid-satellite or satellite III have been isolated, characterised and sequenced on peri-centromeric region of human chromosomes [4,5]. Studies using particular restriction enzyme periodicities and primary nucleotide sequence analysis has revealed that many, if not all human chromosomes are characterised by specific subsets of alphoid satellite DNA [6,7]. The molecular and evolutionary basis for the chromosome specificity of subsets of different satellite DNA, including alphoid satellite, is unclear.

It is believed that the mosaicism is probably more frequently present than expected from classical cytogenetic examinations. FISH analysis of uncultured lymphocytes, amniocytes and chorionic villus cells may in fact aid diagnosis of any suspected constitutional mo-

saicism [8, 9]. The overall aim of the present study was to compare the efficiency of classical cytogenetics and interphase FISH techniques in detection of mosaic form of Turner syndrome.

### Methods

Heparinised peripheral blood samples prepared from 78 patients demonstrating the clinical features compatible with Turner syndrome were used to compare the detection efficiency of standard cytogenetics and interphase FISH methods.

The cytogenetic analysis of samples was carried out according to the standard methods. The metaphase cells were stained by GTG-Banding and studied under a light microscope. About 50 metaphase cells were analysed for each patient where available.

For interphase FISH purposes, 1 ml of heparinized peripheral blood sample obtained from each patient was directly treated with hypotonic solution and fixed on to the microscope slides. The target DNA was denatured in 70% formamide/2×SSC at 70-75°C for 2-3 minutes transferred to chilled 70% ethanol and then dehydrated through ethanol series. The slides were air dried and used for commencing FISH.

The commercially available DXZ1 biotine labelled probe (Q-biogene) hybridising to highly repeated alphoid DNA sequences of chromosome X centromere, was used to enumerate the chromosome X copy number in interphase nuclei. For hybridisation, 0.5-1 ng of biotin labeled DXZ1 was diluted in 10 µl of hybridization buffer and denatured at 65-70°C for 10 minutes. The denatured DNA probe was applied on denatured target, covered by a 22× 22 mm cover slip and sealed with rubber cement. The probe and target DNA were cohybridized by incubation of slides in a water bath at 42°C for 12-16 hours.

The probe detection with fluorescein isothiocyanate (FITC) conjugated to avidin and post hybridization washes were performed according to carter et al. (10). The signals were ampli-

Table 1. Comparison of results obtained from analysis of samples using conventional cytogenetics and FISH methods. Data shown as N (%).

Karyotype	Cytogenetics	FISH
45x	32 (41)	32 (41)
47xxx	3 (3.8)	3 (3.8)
45x/46xx	9 (11.5)	13 (16.7)
45x/46xx/47xxx	2 (2.7)	3 (3.7)
normal	26 (33.3)	27 (34.8)
X-Structural aberrations	6 (7.7)	0 (0.0)
Total	78 (100)	78 (100)

fied once. The cells were counter stained with 0.4 µg/ml 4,6 diamino-2-phenylindol-dihydrochloride (DAPI) and 0.2 µg/ml propidium iodide in mounting medium AF1 (Citiflour Ltd) and evaluated under a conventional fluorescence microscope.

A minimum of 100 interphase cells was analysed for each sample. The cut-off scheme similar to that of Lier et al [11] were used to differentiate between normal, monosomic, disomic and trisomic samples for chromosome X. A sample was considered normal if 93% or more of cells showed two specific signals. A monosomy X result was reported where a minimum of 87% of cells demonstrated a single signal on each cell. Samples simultaneously showing one, two or three signals on a minimum of 40%, 49% and 21% of cells respectively, were defined as mosaics for X chromosome. Data were analyzed using chi-square test and SPSSv.15 as statistical software. A P-value of less than 5% was defined as the significant level.

### Results

Thirty-two samples were detected as 45,X and 3 samples as 47,XXX by GTG-Banding method and confirmed by Interphase FISH. Nine Samples were detected as 45,X/46,XX mosaicism and 2 samples as 45,X/46,XX/47,XXX by conventional cytogenetic studies and then subsequently confirmed by Interphase FISH. Five samples were diagnosed as normal by conventional cytogenetic method, while 4 samples shown to be mosaics form of 45,X/46,XX and the fifth sample detected as 45,XX/46,XX/47,XXX by interphase FISH. Six samples showed structural aberrations for X chromosome, which were undetectable by interphase FISH. The remaining 21 samples revealed to be normal by both cytogenetics and FISH analysis. The results are summarised as percentages in table 1.

As it can be concluded from table 1 using FISH analysis, about 20.4 percent of the samples were correctly shown to be mosaics for X

aneuploidy, while 14.2 percent of the samples diagnosed as mosaics by conventional cytogenetic method. This means that about 6.2% of mosaic cases were failed to be diagnosed by conventional cytogenetic results. In contrast six samples (7.7%) showed structural aberrations of chromosome X by cytogenetic analysis which were undetectable by FISH. No false positive results were obtained using either method in this study.

### Discussion

Turner's syndrome affects about 1/2500 female infants born alive. About 47% of patients show a mosaic form of the syndrome, while the remaining results from total or partial absence of one of the two X chromosomes normally present in females [11]. A significant relationship has been reported between chromosomal anomalies and clinical expression of TS by several studies. It has been shown that mosaicism mitigates the TS phenotype and the cardiovascular risk factor profile [12]. Short stature and primary amenorrhea have been shown to be correlated with total deletion of one chromosome X or imbalanced gene dosage due to structural X anomalies. Whereas cases of infertility, recurrent miscarriages and secondary amenorrhea are associated with a mosaic karyotype pattern (45,X/46,XX or 45,X/46,XX/47,XXX ...), with a slight mosaicism in most cases [13].

Accelerated loss of ovarian primordial follicles from the 18th week of fetal life, resulting in gonadal dysgenesis, characterizes classical Turner syndrome. However, in women with mosaic TS, follicular development can persist beyond puberty, leading to a spontaneous pubertal development, regular menses and even pregnancy before the onset of premature menopause [14]. This indicates the possibility of fertility preservation in young women with gonadal dysgenesis [15]. Examining ovarian tissue histopathologically, Hreinsson et al. [16] noted that follicles exist in most of the ovaries

in these young women. It has also been reported that Mosaics are diagnosed 8 years later than 45,X cases [17]. All of these observations emphasize the necessity for a stricter genotype categorization not only in the clinic but also in research on TS than previously adopted.

The present study was performed on 78 patients with symptoms indicating for TS. The patients' age ranged from one week to 45 years at the time of sampling. The overall objective of our study was to compare the detection efficiency of standard cytogenetics and interphase FISH methods in diagnosis of mosaic form of turner syndrome.

In this study a greater percentage of the mosaic samples were detected by interphase FISH (20.4%) compared to the cytogenetic analysis (14.2%). However the structural aberrations of X chromosome were undetectable by this technique leading to a false negative rate of 7.7%. The conventional cytogenetic technique is capable of detecting all types of numerical and structural chromosome abnormalities in a single experiment, but the sensitivity of the method in diagnosis of mosaic turner syndrome is low (68.75%).

These results indicated that using the two techniques in parallel, a high sensitivity and specificity is achievable in clinical practice for correct evaluation of cytogenetic basis of abnormal clinical features in patients with turner syndrome. Using this strategy will allow the cytogeneticists to correctly differentiate between mosaicism and homogenous karyotype 45,X, where the structural aberrations of the X chromosome which accounts for about 21% [11] of turner patients will not be missed.

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