Detection of low level sex chromosome mosaicism

Abstract

Turner syndrome (TS) is most commonly due to a 45, X chromosome defect, but is also seen in patients with a variety of X-chromosome abnormalities or 45, X/46, XY mosaicism. The phenotype of TS patients is highly variable, and depends largely on the karyotype. Patients are at an increased risk of gonadoblastoma when a Y derived chromosome or chromosome fragment is present. Since constitutional mosaicism is present in approximately 50% of TS patients, the identification of minor cell populations is clinically important and a challenge to laboratories.

Aim: The purpose of the present study was the application of fluorescence in situ hybridization (FISH) assay to identify low level mosaicism for an XY or XX cell population for TS patients with monosomy X and also to identify the nature of sex chromosome markers detected by conventional cytogenetic studies.

Methods: The study included 65 female patients with a clinical suspicion of TS, they were selected from the Genetic Clinic, Medical Research Institute, University of Alexandria. Chromosome analysis by G-banding technique was done. FISH was performed using centromere probes for the X and Y chromosomes.

Results: Chromosome analysis by G-banding technique revealed the following results: twenty patients (30. 77%) had a 45, X karyotype; mosaicism for a second normal or structurally abnormal X was observed in 27 (41. 54%) cases, mosaicism for Y chromosome in 5 (7. 69%) cases, 7 (10. 77) had mosaicism involving a marker chromosome; and non mosaic

structural abnormalities of the X chromosome was present in 6 (9. 23%) patients. To further investigate the possibility of mosaicism in the 20 patients with an apparently nonmosaic 45, X karyotype, and to identify the nature of chromosome markers in the 7 patients carrying a marker, FISH was performed using centromere probes for the X and Y chromosomes. A minor XX cell line was identified in 6 patients, XY mosaicism were identified in 3 cases and the 45, X result was confirmed in 11 samples. FISH analysis performed on the 7 patients with chromosome markers, identified the origin of these markers as X chromosome material in 3 patients, and Y-derived chromosome in 4 patients (idic Y with a double hybridization signal corresponding to double centromeric region).

Conclusion: FISH is a useful tool in the detection of low frequency cell lines and identification of the nature of unknown chromosome markers that have important implications for the management of patients with Turner syndrome. FISH as an adjunct to karyotype analysis provides a sensitive, specific, rapid, and informative technique to identify sex chromosome mosaicism in TS patients.

Key Words: Turner syndrome, monosomy X, mosaicism, 46, XY cell line, gonadoblastoma.

INTRODUCTION

The incidence of Turner syndrome (TS) is approximately one in 3, 000 newborn girls and is associated with an apparently nonmosaic 45, X karyotype in many of these patients.(1) Based on chromosome analysis 30%-50% are mosaic with a second X or a structurally abnormal X, and fewer than

10% of TS patients have mosaicism with a 46, XY cell population or a Y chromosome rearrangement. The mosaic status of the remaining TS patients remains uncertain but of clinical interest because if they do have cells with a Y chromosome or Y-derived fragment, they may have an increased risk of gonadoblastoma.(2)

Because a 45, X karyotype usually causes fetal death, it has been postulated that all liveborn 45, X infants must be mosaic with either a Y or a second X in some cells.(3) The hypothesis of the necessity of mosaicism for survival is supported by the argument for the existence of a feto-protective effect of one or more genes on the sex chromosomes (X or Y). According to this concept, two copies of the gene(s) should be present, either in the fetus or in the extra-embryonic tissues.(4) Both, embryonic mortality and the Turner phenotype, are considered to be a result of monosomy of a common gene (s) of the X and Y chromosomes. It is assumed that, in women, these genes are expressed in both active and inactive X chromosomes as a means of ensuring the right quantity of genetic product.(5)

The American College of Medical Genetics recommends cytogenetic analysis of 30 metaphase cells to rule out sex chromosome mosaicism.(6)

This analysis can identify 10% mosaicism with a confidence level of 95% but a more sensitive level of detection requires analysis of many more metaphase cells, which is costly. PCR-based assays have been used to identify low-level mosaicism.(7) Fluorescence in situ hybridization (FISH) using X and Y chromosome probes has been validated (8) as a reflex test in apparently nonmosaic 45, X individuals to identify low-level mosaicism.(2, 9)

One of the advantages of applying the FISH technique is the possibility of studying mosaicism in both interphase nuclei and metaphases.(5)

The purpose of the present study was to show the value of FISH analysis to identify low level sex chromosome mosaicism in Turner syndrome with nonmosaic monosomy X, and also to identify the nature of marker chromosomes detected by conventional cytogenetic studies.

METHODS

The study included 65 patients with clinical features suggestive of TS, they were selected from the out patient clinic, Human Genetics Department, Medical Research Institute, University of Alexandria over a period of 4 years. Chromosomes were prepared according to standard techniques for culturing lymphocytes from peripheral blood, and the preparation was treated with trypsin to obtain G-banding.(10) A minimum of 30 metaphases were analysed, and 3 were photographed for each patient.

FISH analysis using the classic alpha-satellite probes for the X [DXZ1] and Y [DYZ3] centromeres (CEP-X and CEP-Y, Vysis Inc., spectrum green hybridizes to the centromere of human chromosome X, and spectrum orange hybridizes to the centromere of human chromosome Y) was performed in cases with nonmosaic 45, X karyotype to detect low level sex chromosome mosaicism and also in cases with chromosome markers to identify the nature of these markers. The protocol followed was that provided by the manufacturer. The normal cutoff was determined to be 1. 0% for a second X signal and 0. 6% for a Y signal in analysis of 500 interphase cells. (11) Whenever interphase FISH analysis revealed evidence of a second cell population, a search was

undertaken using FISH for metaphase cells to confirm its presence and examine the structure of the sex chromosomes in that population. FISH was performed in the Genetic Center, Genetic Counseling Society, Alexandria.

Statistical analysis:

Data were presented in the form of frequency and percentages.

RESULTS

Based on G-banded chromosome analysis of a minimum of 30 metaphase cells for the 65 patients included in this study, 45, X karyotype was found in 20 cases (30. 77%), various mosaic complements was detected in 39 (60%), and non mosaic structural abnormalities of the X chromosome in 6 (9. 23%) (table I). Mosaicism detected were as follow: 8 (12. 31%) with numerical mosaicism involving the X chromosome, 19 (29. 23%) with structural mosaicism of the X chromosome, 5 (7. 69%) with Y chromosome mosaicism, and 7 (10. 77%) with mosaicism involving a marker chromosome, the level of mosaicism ranged from 8% to 86%.

FISH analysis, using centromere probes for the X and Y chromosomes, identified mosaicism with a second X chromosome in 6 of the 20 patients with an apparently non-mosaic 45, X karyotype. The level of mosaicism detected ranged from 3. 8% to 8. 2%. Mosaicism with a Y chromosome was detected in 3 patients, the level of mosaicism ranged from 2. 4% to 7. 2% (table II) (figure1). FISH improved the identification of mosaicism from 60% (39/65) to about 73. 85% (48/65).

FISH analysis performed on the 7 patients with chromosome markers, identified the nature of these markers as X chromosome material in 3 patients, and Y-derived chromosome in 4 patients (idic Y with a double hybridization signal corresponding to double centromeric region) (table III).

FISH highlighted the differences between the initial diagnosis, based on G-banding, and the final diagnosis, determined by specific probes for the X and Y chromosomes. FISH analysis detected more Y-chromosomal material than karyotyping (18. 46% (12/65) vs. 7. 69% (5/65), respectively), and also detected more X-chromosomal mosaicism among the TS patients (55. 38% (36/65) vs. 41. 54% (27/65), respectively).

Clinical, ultrasound and laparoscopic examination of gonads in patients with Y chromosome material revealed normal females with bilateral rudimentary streak gonads in 9 patients and females with clitoromegaly, unilateral streak gonads, and contralateral intraabdominal testis in 3 patients.

- Interphase cells showing one green signal of the X chromosome
- Interphase cell showing 2 green signals for X chromosome
- Interphase cell with one green signal for X chromosome and one red signal for Y chromosome
- Metaphase cell with one green signal for X chromosome and one red signal for Y chromosome

DISCUSSION

An estimated 1 in 50 conceptuses is affected with TS. However, only 1% of TS conceptuses survive to birth. It has been observed that there is a higher ratio of mosaic karyotypes to monosomy X in live births compared to aborted

fetuses. This finding has led to speculation that most if not all patients born with TS must have mosaicism.(3)

Phenotypic expression in TS patients largely depends on the karyotype, and identification of sex chromosome mosaicism plays a key role in clinical management. Patients with documented mosaicism for a 46, XX or duplication of the long arm have a moderate phenotype. Mental retardation is seen more frequently in patients with a small ring chromosome and deletion of the X-inactivation center (XIST).(12) Patients with a Y or Y-derived chromosome identified by routine G-banding analysis may have as high as a 30% risk of developing gonadoblastoma, although most reports suggest an incidence of 7%- 10%. Therefore, identification of low – level Y chromosome mosaicism is also clinically important.(13)

Out of the 65 patients included in the present study, 45, X karyotype was found in 20 cases (30. 77%), various mosaic complements was detected in 39 (60%), and non mosaic structural abnormalities of the X chromosome in 6 (9. 23%). Previous studies reported constitutional mosaicism in approximately 50% of TS patients based on chromosome analysis.(14) The detection of mosaicism is mainly influenced by the type and number of tissues analysed, the number of cells studied, and the sensitivity of the techniques applied.(4, 5, 15)

FISH analysis of the 20 patients with 45, X karyotype included in the present study detected mosaicism in 9 patients: 6 had an XX cell line, and 3 had Y chromosome material. Therefore, FISH improved the identification of mosaicism from 60% to about 73. 85%. Van Dyke and Wiktor (11) reported

that FISH analysis improves the identification of mosaicism from 55% to 67% in patients with nonmosaic 45, X karyotype. They concluded that the identification of a cell population with a second X chromosome is sufficient to exclude, with a high degree of confidence, the presence of a Y-bearing cell population in that patient. Other investigators compared the results of lymphocyte G-banded karyotype with the use of interphase X/Y FISH analysis. They detected more Y-chromosomal matrial by FISH than karyotyping (in 15% vs. 11% of the women, respectively) and also detected more X-chromosomal mosaicism among the TS women (in 70% vs. 45% of the women respectively). They suggested the use of X/Y interphase FISH as a complement to karyotyping in order to obtain a more complete knowledge of the chromosome constitution of each individuals with TS.(16)

The Y-chromosomal material in TS individuals is often present in the form of small marker chromosomes, which are difficult to positively identify by routine karyotyping. Furthermore, small markers are frequently missed altogether using this technique, especially if limited numbers of metaphases are evaluated.(17) In the present study, 7 (10. 77%) patients were detected with mosaicism involving a marker chromosome, FISH analysis identified the origin of these markers as X chromosome material in 3 patients, and Y-derived chromosome in 4 patients, the nature of the Y chromosome was defined as isodicentric with two centromeres. Approximately 20% of mosaic patients with TS have a sex marker chromosome.(14) The use of fluorescence in situ hybridization (FISH) analysis has been well documented as being effective in detecting and identifying sex chromosome markers.(18, 19, 20)

In the present study, final diagnosis followed G-banding and FISH analysis identified Y-bearing cell population in approximately 18% of TS patients. Review of the literature suggested that 6-12% of patients with TS had 45, X/46, XY cell lines with or without structurally abnormal Y.(21) This variation is a reflection of the numbers of patients studied, the technique used, and the strategies employed by different investigators to search for small populations of Y containing cells.

Virilization with clitoromegaly was found in 3 cases in the present study with Y cell line mosaicism. It is believed that virilization in patients with TS is due to the presence of Y cell line within the gonad even if the Y cell line is not identified in peripheral blood. Therefore, virilization is an indication for detailed studies looking for the presence of Y mosaicism. (22)

Early detection of Y-derived material in the genome of TS individuals is of great importance because of the relative high risk (10-30%) of developing gonadal tumors (i. e., gonadoblastoma or dysgerminoma).(23, 24) Gonadoblastoma is a precursor tumor which may undergo malignant transformation into one of the virulent germ cell neoplasms (dysgerminoma, embryonal carcinoma, endodermal sinus tumor, chorioepithelioma or yolk sac tumor).(21) It has been suggested that a locus (GBY) predisposing to the development of this tumor is located in the pericentromeric region of Yp.(25) Although the natural history of gonadoblastoma in prepubertal patients is unknown, this tumor can be evident even in the first decade of life in streak gonads with Y mosaicism and may be bilateral. Therefore, prophylactic gonadectomy should be recommended in patients with TS and Y

chromosome mosaicism, because fertility is not an issue, surgical morbidity is minor, and the potential for malignant transformation is unknown.(26)

If a patient declines gonadectomy, monitoring for germ cell neoplasm is the only option. However, it is unclear whether methods in common use today (vaginal ultrasound, biochemical markers, proteomics, etc.), even with compliant patients, are able to identify germ cell neoplasms at early enough stages to improve the natural history of the disease. It is possible that a predictable and specific marker of malignant potential may be identified in the future. Until then, physicians will need to be continually updated on these important issues as they relate to the clinical management of patients with Turner syndrome.(21)

In conclusion, FISH for the X and Y centromere probes is a useful adjunct to conventional cytogenetic studies in patients with apparently nonmosaic monosomy X. This additional assay improved the identification of mosaicism from 60% to about 73. 85%. FISH method provides a sensitive, specific, rapid, and informative means of identifying low level X and Y mosaicism in TS patients, and can be employed on the same blood sample that is used for the conventional cytogenetic studies. FISH helped in identifying the nature of the unknown markers which has an important implication in the development of gonadal tumors. Metaphase FISH, and interphase FISH should complement and validate each other in the detection of covert Y and identification of rearranged X vs Y chromosomes.