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Research Paper

Y Cell Line in a Turner Mosaic: A Case Report

Medical Science

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ABSTRACT

Background: Turner Syndrome is one of the most common chromosomal aneuploidy seen in humans with an incidence of about 1: 2500 newborn females. Approximately 60% patients with Turner syndrome have 45, X karyotype while others show X chromosome abnormalities like deletions of long arm or short arm, isochromosome or ring chromosome. About 6-9% cases also show presence of Y chromosome or Y derived sequences. Turner Syndrome patients with ovarian dysgenesis and Y Cell- line / Y derived sequences have higher risk of developing gonadal tumors. In the present study we report on a patient with Turner Syndrome showing mosaicism with Y cell line.

Case Report: We report a 19 yrs old unmarried female who reported to Gyn OPD with primary Amenorrhoea. She was referred to our lab for cyto-molecular genetic analysis. Routine karyotype was done using standard protocol for Giemsa trypsin banding. Polymerase chain reaction (PCR) for SRY gene was also carried out to identify presence of Y cell line or Y derived sequences. The Cytogenetic results were confirmed by Fluorescent In Situ Hybridization (FISH) using probes for chromosomes X and Y.

The investigations revealed the patient to be a Turner mosaic with karyotype 45, X in 65% cells, 46, XX in 10% cells; and the remaining 25% of the cells showing presence of Y- chromosome with karyotype 46, XY.

Conclusion: The detection of Y-cell line is important in view of 10-30% higher risk of developing gonadal tumors. Prophylactic gonadectomy is recommended to patients of Turner syndrome with Y-chromosome mosaicism and ovarian dysgenesis.

Introduction

Turner syndrome characterized by short stature, webbing of neck, shield like chest and increased carrying angle is the most common aneuploidy syndrome with an approximate incidence of 1: 2500 newborn females.[1] Almost 99% of the pregnancies with Turner syndrome abort spontaneously during the first trimester of the pregnancy.[2] Approximately 60% of all Turner syndrome show 45, X karyotype, 5-10% show X chromosome abnormalities like deletions of long arm or short arm, isochromosome or ring chromosome while remainder show mosaicism for 45, X with one or more additional cell lineages. About 6-9% of Turner mosaic cases show presence of Y chromosome or Y derived sequences.[3, 4]

Dysgenetic gonads serve as a risk factor for origin of germ cell tumors. The precursor lesion is gonadoblastoma a benign tumor which has 60% higher risk of converting into the invasive dysgerminoma in presence of the Y chromosome or Y derived sequences.[5] The gonadoblastoma gene locus has been mapped to pericentric region of the long arm of the Y chromosome.[6] Prophylactic gonadectomy is recommended to patients of Turner syndrome with gonadal dysgenesis showing Y chromosome mosaicism or presence of Y derived sequences.[7]

Growth failure is a consistent finding at birth in infants with Turner syndrome. However, the time of onset and pattern of growth deficiency is unknown.8 Turner syndrome patients generally present with delayed menarche and lack of development of secondary sexual characters.

Conventional cytogenetic analysis is the most common test carried out for patients with primary and secondary amenorrhea, query Turner syndrome and looking for presence of Y chromosome. In many of the Turner mosaic cases, Y-chromosome may be present in a small percentage of cells which may be missed out. Several studies have unanimously supported the use of a sensitive PCR technique to identify the cryptic Y-chromosome.[9]

Fluorescent in situ hybridization (FISH) is a molecular cytogenetic technique which can help in identifying the precise percentage of Y chromosome or its derivatives as large numbers of cells can be screened by this method.

Therefore, the present study was planned to evaluate the patient using both conventional cytogenetic study (karyotype) and molecular based studies (FISH and PCR).

In the present study we report a Turner Syndrome mosaic with Y cell line.

Case report

A 19 yrs old unmarried female reported to Gynaecology OPD of our hospital with complaints of absence of menstrual cycle. She also complained of poorly developed breasts as compared to her classmates. She was 4'1" in height and was shortest amongst girls of her age group. A detailed pedigree info was collected and it was found that nobody amongst her siblings and family tree has similar complaints. A physical examination revealed weight of 39kgs, height 125cms, breast Tanner grade II and pubic hair Tanner grade III. There was no obvious web neck and cubitus valgus deformity. Vaginal examination revealed normal female genitalia with cervix and uterus. Harmonal assay showed elevated FSH and LH levels of 25.8 IU/l and 15.9 IU/l respectively. USG pelvis was suggestive of normal cervix and uterus with bilateral dysgenetic gonads. She was referred to our lab for cyto-molecular genetic analysis.

First 8 ml of venous blood was collected; 5ml in a heparin for cytogenetic analysis and 3 ml in EDTA vials for DNA analysis after taking informed consent. The lymphocytes were cultured in RPMI 1640 and processed for Typsin- Giemsa banding as described previously.[10] The slides were viewed under an Olympus BX61 bright field microscope. Almost 20 metaphase spreads were scored for the individual and analysis was done using the Applied spectral imaging (ASI) software (ASI, Israel).

Polymerase chain reaction (PCR) for SRY gene was also carried out in this case to identify presence of Y cell line or Y derived sequences. Genomic DNA was isolated from the blood samples collected in EDTA by using standard protocol and then subject-

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ed to PCR amplification of SRY gene as described previously.[11]

FISH was further carried out in the patient to reconfirm precise percentage of Y cell line. The cell suspension was dropped on to two slides and areas marked. Ten microlitres of X and Y chromosome probes (SE X/Y, Kreatech, Nethererlands) was applied and hybridized. The slides were counterstained with 0.05mg/ml of DAPI suspended in antifade solution (Vectashield, Vector Labs, UK) and viewed under an Olympus BX61 epifluorescence microscope using appropriate filters. Approximately 200 cells were analyzed for the patient to score the signals. Green signals denoted the presence of X chromosome, and red signals presence of Y chromosome. Analysis was done using software from Applied Spectral Imaging (Isreal).

The cytogenetic analysis confirmed Turner syndrome. The patient was a Turner mosaic with karyotype 45,X in 65% and 46,XX in 10% of the cells (Fig 1a & 1c); the remaining 25% of the cells showed presence of **Y- chromosome** with **karyotype 46,XY** (Fig 1 b).

PCR analysis showed amplification for SRY gene (Ladder 5 of Fig 2). No SRY gene magnification is seen in negative control (Ladder 2 of Fig 2) and another case of Turner with 100% 45,X karyotype (Ladder 3 of Fig 2).

FISH analysis: The same case was assessed by FISH to reconfirm presence of Y- chromosome which is denoted by the red signal (Fig 3).

Discussion:

Turner syndrome seen in approximately 1 in 2500 live born girls generally present with delayed menarche and lack of development of secondary sexual characteristics or with severe/significant short stature. Almost half of Turner syndrome patients show 45, X Karyotype, about 10% show X chromosome anomalies like Xq deletion, isochromosome and ring chromosome while the rest show various percentages of mosaicism with one or more additional cell lineages.[4] About 6-9% of Turner mosaic cases show the presence of Y cell line or overt Y derived sequences.[9]

Turner Syndrome patients with gonadal dysgenesis who show presence of Y chromosome or Y derived sequence have higher risk of developing gonadal tumors. Though gonadoblastoma is a benign tumor there are 60% chances of it converting to malignant dysgerminoma.[5] The gonadoblastoma gene has also been mapped to the pericentric region of the Y chromosome.[12]

Virilization in patients with Turner syndrome indicates the presence of Y- cell line within the gonads; even if Y- cell line is not detected in the peripheral blood by karyotype.[6] It has been recommended that a search for Y-chromosome sequences in Turner syndrome must be performed in all cases of virilization.[13] In our patient there were no signs of virilisation. Since absence of virilisation does not rule out absence of cryptic Y sequences PCR was carried out in our patient which showed amplification for the SRY gene.

Early detection of Y-chromosome cell line or Y derived sequences in Turner Syndrome is of great importance because of high risk of gonadal tumor development.[14] Though the occurrence of Y derived sequences in Turner Syndrome is low, it should be screened meticulously by molecular techniques like PCR.[9, 15] In our study Y derived sequence was detected by PCR as mentioned before. The biochemical parameters of the patient like FSH and LH were raised. The USG pelvis also showed presence of bilateral streak gonads.

Since most studies have indicated 30-35% incidence of Germ Cell Tumors, prophylactic gonadectomy should be offered to Turner Syndrome patients with Y-chromosome or showing presence of Y derived sequences.16 Some have recommended detailed vaginal sonography with color doppler sonography of gonads at regular intervals in these patients however they conclude that gonadectomy is still the procedure of choice to exclude malignancy with absolute certainty.17 Since our patient has streak gonads with Y-Cell line she will be an ideal candidate for prophylactic gonadectomy.

The FISH analysis of more than 200 cells also showed 25% Y-cell line in congruence with conventional karyotype which studied only 20 metaphase spreads. Hence FISH in this case did not provide much additional information.

Conclusion:

The role of Y chromosome in development of Gonadal tumors is still controversial. However several studies have confirmed a higher risk of developing germ cell tumors in Turner Syndrome patients with gonadal dysgenesis showing Y cell mosaicism or hidden Y derived sequences. Hence, all Turner Syndrome patients should be screened for presence of Y chromosome by molecular methods so that prophylactic gonadectomy can be recommended in case of identification of Y- cell line.

In conclusion, it is emphasized that all Turner syndrome patients be screened for the possibility of developing gonadal lesions, either tumoral or not, using both the conventional cytogenetic analysis and the highly sensitive PCR which is also a low cost and easy to perform technique as compared to FISH which is not only expensive but is also labour intensive to prevent occurrences of gonadal lesions.

Legends:

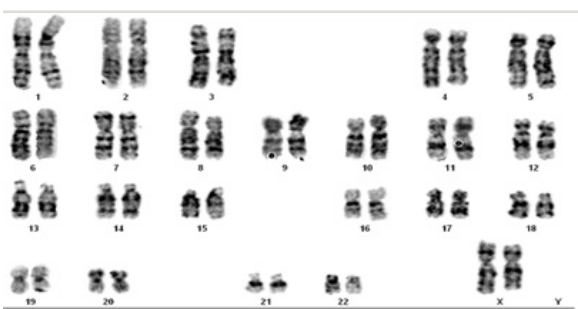

Fig 1a: Karyotype 46, XX

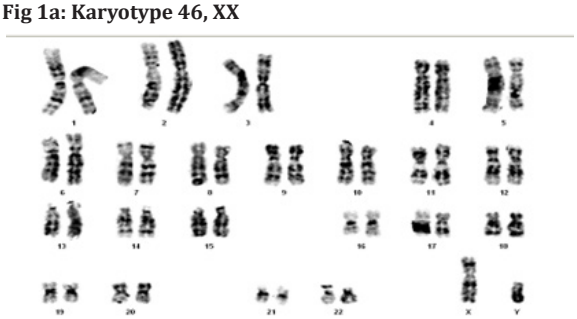

Fig 1b: Karyotype 46, XY

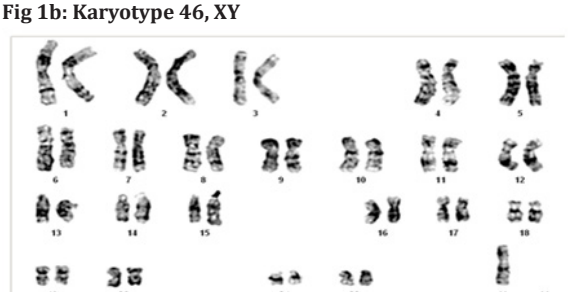

Fig 1c: Karyotype 45, X

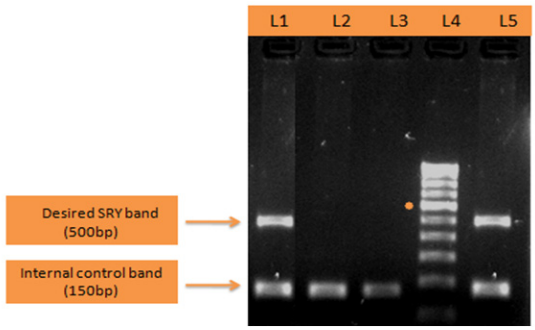

Fig 2: Gel picture showing amplification of SRY gene in Turner mosaic patient

L1: Positive control, L2: Negative control, L3: Patient with 100% 45,X karyotype,
L4:100bp ladder, L5: Turner mosaic showing amplification of SRY gene

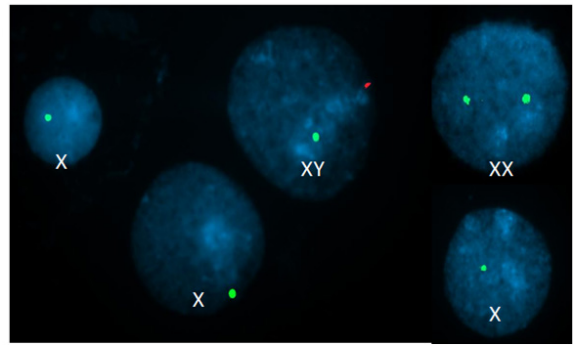

Fig 3: FISH ANALYSIS: Turner Mosaic patient who showed presence of SRY gene by PCR detected to have 3 cell lines by FISH – 45, X; 46, XX and 46, XY

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