Case Report

Unravelling the Impact of an Additional Sex Chromosome in an Adult Female

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Abstract

Women with Triple X syndrome (TXS) appear to be at increased risk for decreased ovarian reserve; however, available data are limited. We present an asyndromic adult female with features of recurrent pregnancy loss and decreased ovarian reserve detected with mosaic Triple X syndrome (TXS). The patient was initially evaluated by a low-cost peripheral blood (PB) conventional karyotyping using standard cytogenetic protocols. Interphase fluorescence *in situ* hybridisation was performed to confirm the diagnosis. Chromosomal microarray, which is a more expensive test, substantiated the presence of additional X chromosomes but failed to detect the presence of low level of mosaicism. Our case study emphasised the recommendation of performing a strategy-based cost-effective cytogenetic evaluation of all cases of decreased ovarian reserve or low anti-Müllerian hormone levels in a resource-constrained setting. It also highlighted the need for additional research to understand the natural history of ovarian function in TXS affected women throughout their lifespans.

Keywords: *Cytogenetic analysis, decreased ovarian reserve, mosaicism, triple X syndrome*

Introduction

Triple X or Triple X syndrome (TXS) is the most common but underdiagnosed sex chromosome disorder present at birth in females, appearing approximately in 1 in 1000 live female births. Due to variations in clinical phenotype and the existence of mosaicism, only approximately 10% of cases are confirmed clinically during their lifetime.[1] The affected females have an extra copy of the X chromosome, leading to a 47,XXX chromosomal presentation. In most of the cases, the affected individuals are physically normal, with a slight increase in the frequency of minor medical and psychological disorders found.[2] A small but marginally enhanced risk of having chromosomally abnormal XXX or XXY children has been demonstrated.[3] Since the initial description in 1959 by Jacobs *et al*., there have been case reports that describe the occurrence of premature ovarian insufficiency (POI) in women with TXS; however, there is a scarcity of data on its prevalence.[4] The cytogenetic abnormality was initially described as 'superfemale', which illustrates a misconception about the syndrome.[5]

The present case study report describes the clinical and cytogenomic profile of a female with recurrent pregnancy loss (RPL) highlighting the association between the decreased ovarian reserve and TXS.

Case Report

A 34-year-old female reported complaints of an inability to conceive for 2 years despite having regular unprotected sexual activity. She attained menarche at the age of 12 years and subsequently had a regular menstrual cycle, and her intellectual and social behaviour appeared to be normal. Married for 9 years and she had conceived earlier three times; two of them ended with early pregnancy abortions, and one

Received: 19-04-2023 **Revised:** 31-05-2023
Accepted: 31-05-2023 **Published:** 30-06-2023

Access this article online

Quick Response Code:

Website: www.jhrsonline.org

DOI: 10.4103/jhrs.jhrs_49_23

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How to cite this article: George S, Dagar V, Chakrabarty BK, Nagaraja N. Unravelling the impact of an additional sex chromosome in an adult female. J Hum Reprod Sci 2023;16:166-9.

pregnancy was diagnosed to be an ectopic pregnancy for which a right salpingectomy was done. She is a known case of type 2 diabetes mellitus on medication, and her blood sugar levels are under control. There was no family history of infertility or RPL. On examination, her height was 162 cm, and her weight was 70 kg. She had no signs of hyperandrogenism or thyroid swelling, signs of galactorrhoea or any acanthosis nigricans. Her blood pressure was within the normal range. Infertility evaluation with hormonal and ultrasound investigations revealed her to have decreased ovarian reserve. The investigation details are shown in Table 1. Her invasive investigations for infertility with diagnostic hysterolaparoscopy showed 2 cm × 3 cm subserosal and intramural fibroids with bilateral tubal block. The semen parameters of her husband were within the normal limit. The patient was planned for *in vitro* fertilisation with embryo transfer (IVF). Conventional karyotyping was performed on the PB samples using standard cytogenetic protocols. For each sample, 50 GTG banded (G banding with trypsin using Giemsa stain) metaphases were analysed. An automated karyotyping system (MetaSystems, GmbH, Altlussheim, Germany) was used for analysis and reported according to the International System for Human Cytogenomic Nomenclature (version: 2020). Cytogenetic analysis of 50 metaphases from phytohaemagglutinin-stimulated cultures using PB sample showed a mosaic karyotype with two cell lines. One cell line (45 metaphases) showed 47 chromosomes due to trisomy X and the other cell line (5 metaphases) showed 46 chromosomes. Fluorescence *in situ* hybridisation confirmed the diagnosis and presence of mosaicism with three hybridisation signals (SG) for the X chromosome in 90% of interphase cell nuclei out of the 500 cells studied. Chromosomal microarray analysis (CMA) was performed using an Affymetrix CytoScan™ 750K array. CMA supported the presence of an abnormal female chromosome complement, including the gain of one complete X chromosome, but it failed to detect a low level of mosaicism [Figure 1]. A combination of two culture system analyses, i.e. RPMI media 1640 containing 5% foetal bovine serum added with inducers 5-fluoro-2'deoxyuridine and methotrexate excluded presence of fragile X syndrome cytogenetically. One hundred metaphases were screened and cut off value as presence of ≥4% metaphases with fra (X) was used to exclude fragile X syndrome.

Table 1: Relevant clinical and laboratory parameters

Parameter	Results
Height (cm)	162
Weight (kg)	70
BMI	26.7
Blood group	B positive
Hb % (g/dL)	12.8
ESR	16 mm/1st h
FSH (RR: 3.08–8.08) (IU/L)	4.15
LH (RR: 1.04–15.0) (IU/L)	4.52
Prolactin (RR: 4.07–24.4) (ng/mL)	11.66
AMH (RR: 3.19–3.95) (ng/mL)	1.15
T3/T4/TSH	WNL
β2 glycoprotein	Negative
Lupus anticoagulant/antinuclear antibodies	Negative
Anticardiolipin antibodies	
IgM	Positive
IgG	Negative
Mantoux test	Negative
LFT/RFT	WNL
HbsAg/HIV/anti HCV	Non-reactive

HbsAg=Hepatitis B surface antigen, Hb=Haemoglobulin, ESR=Erythrocyte sedimentation rate, LFT=Liver function test, RFT=Renal function test, AMH=Anti-Müllerian hormone, FSH=Follicle-stimulating hormone, LH=Luteinising hormone, BMI=Body mass index, TSH=Thyroid-stimulating hormone, IgM=Immunoglobulin M, IgG=Immunoglobulin G, HCV=Hepatitis C virus, WNL=Within normal limit, RR=Relative risk

The couple was counselled about the cytogenetic diagnosis and the possibility of a recurrence of abortion and its effect on the subsequent child. The couple was also counselled on the requirement of oocyte donation for a good outcome. However, as the facility of oocyte donation was not available in the treating ART centre and the couple did not want to go to the ART bank or other ART centres for personal reasons, they opted to go through the IVF with self-gametes. IVF treatment was started with the antagonist protocol, and ovum pickup was done, which yielded two oocytes, but these oocytes did not get fertilised. The couple was again counselled about the failure of the IVF procedure and the benefits of IVF with the donor oocytes. The couple got convinced and underwent IVF with donor oocytes and she is presently at 16 weeks of pregnancy.

Discussion

In 90% of 47,XXX conceptions, the additional X chromosome is derived from maternal non-disjunction during meiosis I, which has been linked to increased maternal age comparable to other chromosomal aneuploidies.[6] Numerical abnormalities in the chromosomes of the gamete result in meiotic errors, leading to an imbalance in the embryo/foetus. These imbalances may result in live births with congenital abnormalities or may even be lethal to the foetus or embryo culminating in pregnancy loss.[7]

Previous studies showed 3% of women had detected POI present with a 47,XXX karyotype; however, the

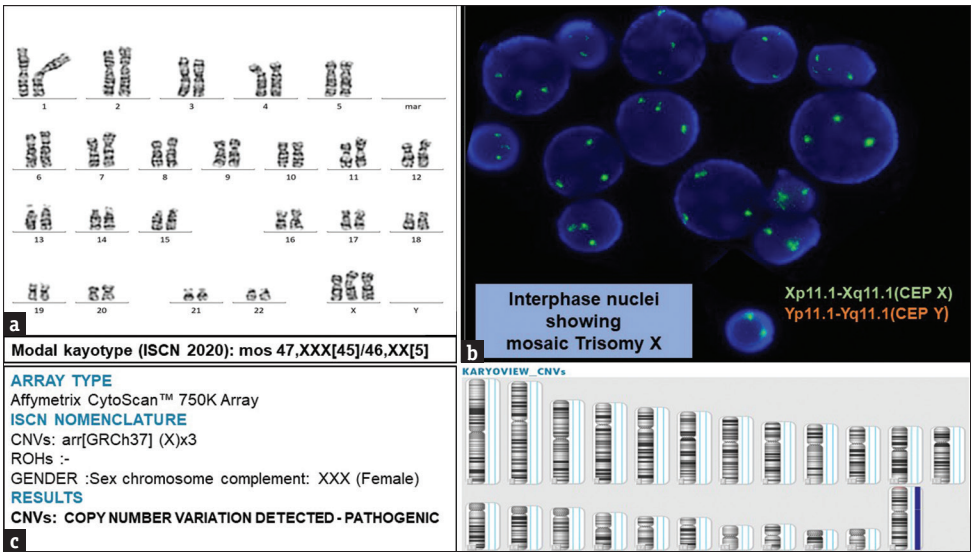

Figure 1: (a) Karyogram showing 47 chromosomes due to trisomy X, (b) FISH analysis with centromeric XY probe on interphase cells showing presence of mosaicism with some cells exhibiting three hybridisation signals (SG) for X chromosome admixed with some cells exhibiting two hybridisation signals (SG) for X chromosome, (c) CMA showed a gain of chromosome X from X pter to qter, indicating trisomy for this chromosome. FISH = Fluorescence *in situ* hybridisation, CMA = Chromosomal microarray analysis

prevalence of ovarian malfunction amongst TXS is unknown. Approximately 2% of Turner syndrome cases have a 45, X/47,XXX mosaic karyotype, and they are phenotypically mildly affected.[8] Studies also recommended anti-Müllerian hormone (AMH) as a potential biomarker of the ovarian follicular reserve. Overall, the studies highlighted the association between TXS and ovarian dysfunction; however, the data are limited.[9] Therefore, a strong and significant association between TXS and POI or low AMH levels has yet to be established. It is also important to mention that previous studies also showed, there are other factors that can serve as biomarkers for these conditions, such as fragile X syndrome and single nucleotide polymorphisms on genes such as BMP, AMH and AMHR. In this study, we have performed only karyotype analysis to exclude the presence of fragile X syndrome (FXS). However, polymerase chain reaction and Southern blot analysis are recommended to exclude FXS which potentially helps establish a stronger relationship between TXS and the conditions.

The International Standards for Cytogenomic Array Consortium highlighted limitations of the costly chromosomal microarray as it cannot detect low-level mosaicism. In our study, we also found a similar incidence.[10] Cytogenomic workup for cases of decreased ovarian reserve is still an uncommon practice in India, due to a lack of awareness and limited infrastructure. Therefore, a strategy-based cost-effective cytogenetic analysis may help the treating physician counsel the patient about the likely transmission of the genetic disorder to the offspring and the likely bad outcome of pregnancy even after conception. Such patients may be counselled for oocyte donation IVF, which is expected to give a good result, or for IVF with own eggs and pre-implantation genetic testing for aneuploidy in view of the mosaicism.

The primary aim of this case study is to emphasise the recommendation of performing a cytogenomic evaluation of all cases of decreased ovarian reserve or diminished AMH level so that we do not miss out on the diagnosis of the rare causes of sex chromosomal aneuploidies having prognostic and therapeutic significance.

Consent

Written informed consent has been obtained from the patient for the publication of case details.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patient has given her consent for her images and other clinical information to be reported in the journal. The patient understands that her name and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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