Trisomy X syndrome is a sex chromosome aneuploidy with a variable clinical presentation at different stages of life. We present two asyndromic females with primary infertility and features of premature ovarian failure (POF). The first case was a nonmosaic trisomy X with poor ovarian reserve on pelvic ultrasound and elevated gonadotropins, while the second case was a mosaic trisomy X who had partly preserved ovarian reserve with normal gonadotropins. The 47,XXX syndrome is a relatively uncommon presentation of POF, leading to infertility and can be missed clinically because of its variable presentation. Therefore, we suggest that genetic testing should be a part of early workup in young women presenting with primary infertility and POF for detecting chromosomal aneuploidies, which will require genetic counseling and alter the management.

Keywords: Premature ovarian failure, primary infertility, trisomy X

INTRODUCTION

Trisomy X syndrome is not a rare but an underdiagnosed sex chromosome aneuploidy of the “X” chromosome, in which females have an additional copy of X chromosome, leading to 47 chromosomes (47, XXX) number, as compared to the 46, XX in a normal female. This condition was first documented in a 35 years old, described as a “superfemale,” with secondary amenorrhea. This syndrome has a frequency of 1/1000 live births, but only 10% of individuals are diagnosed in their lifetime. In 90% of cases, the extra X chromosome is derived from maternal nondisjunction during meiosis I, which has been linked to advanced maternal age similar to other chromosomal aneuploidies.

There is wide variation in phenotype, which may manifest at different stages of life. Most of the patients at birth do not have any obvious dysmorphic features except low birth weight. During the neonatal period, subtle facial dysmorphism, hand and feet abnormalities, and epicanthal folds which may give a clue. Most of the school-going girls have variable stature, while some may have attention deficit, mood disturbances, or other psychiatric illness. Pubertal onset and secondary sexual characters are usually normal. Fertility is usually normal in most cases with successful pregnancy outcomes unless complicated by premature ovarian failure (POF), a condition in which ovarian functions are impaired before the normal age of menopause. The present study reports the clinical, hormonal, and cytogenetic profiles of two nulliparous females attending the fertility clinic of a tertiary care center in Maharashtra, India.

METHODS

The patients were interviewed about relevant medical, reproductive, and family history. Quantitative hormonal profile was done from blood sample by chemiluminescence immunoassay. Chromosomal analysis (CA) was performed on phytohemagglutinin-stimulated peripheral blood T-lymphocytes using GTG banding. CA was done using image processor (CytoVision) version 7.2. Chromosomal abnormalities were reported...
according to An International System for Human Cytogenomic Nomenclature (ISCN, version: 2016). Minimum twenty metaphases were analyzed with additional thirty metaphases on suspicion of low-level mosaicism. Fluorescence in situ hybridization (FISH) was performed for mosaic karyotype using IVD-approved fluorescence-labeled X-centromeric DNA probes according to manufacturer’s (MetaSystems) instructions.

**Results**

**Case 1**
A 30-year-old female, married and cohabiting for the past 6 years, presented with primary infertility. She attained menarche at 13 years and had regular cycles (3–5/30 days) till the past 15 months when she developed oligomenorrhea.

CA revealed a female chromosomal constitution with an additional X chromosome in all twenty metaphases, ISCN result: 47, XXX[20] [Figure 1].

**Case 2**
A 29-year-old female, married for 4 years and cohabiting since then, presented also with primary infertility. She attained menarche at 12 years and had regular cycles (3–5/28 days) but subsequently developed oligomenorrhea for the past 2 years.

CA revealed a mosaic karyotype with an additional X chromosome in 11 metaphases, ISCN result: 47, XXX[11]/46, XX[39]. This was confirmed on FISH with 47, XXX chromosomal constitution in 24.6% of interphase cell nuclei out of 500 cells studied [Figure 2].

Both females had a normal systemic examination, sexual maturity rating, and no dysmorphism. They had normal intellectual and social behavior during childhood. Two-dimensional echocardiography, hematological, biochemical parameters, thyroid function tests (T3, T4, and thyroid-stimulating hormone), and thrombophilia profile (protein-C, protein-S, and antithrombin III) were in a normal range. Diagnostic laparoscopy revealed patent tubes. Semen analysis of the husbands was normal with unremarkable family history.

Relevant clinico-hormonal profiles of both cases are compared in Table 1.

**Discussion**
In triple X Syndrome, two of the three X chromosomes are inactive. The genes located at the pseudoautosomal regions (PARs) of X chromosomes and another 5%–10% of genes outside the PAR regions, escape X-inactivation and remain genetically active. This hypothesis can explain the variable phenotypic features in these patients and further lead to underdiagnosis in many cases.[7]

Both females were slightly taller and above the 75th percentile for height, which is in concurrence with a larger study conducted by Ottesen et al., in which they found taller females ranging from 1 to 3 Standard Deviation score (SDS) of the average population.[8] Our patients did not have any dysmorphic features. They had normal motor development, social conduct, and optimal linguistic skill. There was no history of major medical issues such as cardiac anomalies or anatomical defects in genitourinary tract. Other studies have also reported variable phenotypic features in these individuals at different phases of life.[3,4] Hence, apart from reproductive problems, both our patients were clinically normal.

In our study, we had one nonmosaic and one mosaic female with trisomy X. Nonmosaic 47, XXX chromosomal constitution is the most frequent abnormality. Mosaicism occurs in approximately 10% of cases and can exist in many combinations such as 46, XX/47,XXX or 46, XX/47,XXX/48,XXXX, or as mixed karyotypes in Turner syndrome such as 45,X/47, XXX or 45, X/46, XX/47, XXX.[9]
Although fertility in females with trisomy X is generally preserved with functional reproductive span, there is an increased risk for POF in these cases. Monosomy X (Turner syndrome) both in mosaic and nonmosaic forms; deletions or translocations in the critical region between Xq13 and Xq26 and premutation in FMR1 gene are more common genetic causes responsible for POF in 20%–25% of cases. There are case reports on the patients who presented with POF with endocrine abnormalities in trisomy X syndrome.[10] Both cases were young and had oligomenorrhea with interesting hormonal profile [Table 1]. The first case with nonmosaic trisomy X had elevated gonadotropins and low anti-Müllerian hormone (AMH) level with absent follicular activities in bilateral ovaries, whereas the second case with mosaic trisomy X had lower normal range of AMH with normal gonadotropins and suppressed follicular activity in solitary ovary. Hence, the nonmosaic form had a more deranged hormonal profile with poor ovarian follicular reserve as compared to the mosaic form. Both our cases, postgenetic counseling, had conceived after successful ovum donation.

### Conclusion
In a young phenotypically normal female with primary infertility, secondary amenorrhea, and deranged hormonal (gonadotropins and AMH) profile, the treating gynecologist should have a high index of suspicion for genetic abnormalities, and a cytogenetic testing must be considered at an early stage of workup to rule out sex chromosomal aneuploidies for appropriate management.

### Ethical approval
Institutional Ethical Committee approval (IEC S. No.IEC/2020/80) and informed consent was obtained from the patients for publication of these case reports.

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

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### Conflicts of interest
There are no conflicts of interest.

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### Table 1: Comparison of clinico-hormonal profiles of cases

| Parameter                      | Case 1                | Case 2                |
|-------------------------------|-----------------------|-----------------------|
| Height (cm)                   | 169                   | 164                   |
| Weight (kg)                   | 72                    | 69                    |
| BMI (kg/m²)                   | 25.2                  | 25.6                  |
| FSH (reference range: 3.08-8.08 IU/L), IU/L | 19.11                 | 4.74                  |
| LH (reference range: 1.04-15.0 IU/L), IU/L | 17.2                  | 2.67                  |
| Prolactin (reference range: 4.07-24.4 ng/ml), ng/ml | 7.46                  | 10.15                 |
| AMH (reference range: 3.19-3.95 ng/ml), ng/ml | 0.03                  | 2.9                   |

Transvaginal ultrasound

| Normal uterine study. Right ovary: 2.7 cm×1.6 cm×1.8 cm | Normal uterine study. Right ovary: 2.1 cm×1.7 cm×1.9 cm with no follicular activity |
| Left ovary: 2.0 cm×1.1 cm×1.0 cm | Left ovary measured 2.4 cm×1.3 cm×1.2 cm with few small follicles |

AMH=Anti-Mullerian hormone, LH=Luteinizing hormone, FSH=Follicle-stimulating hormone, BMI=Body mass index
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