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Fraccaro Syndrome (49,XXXXY): Case study in antenatal and postnatal from South Indian patients, an awareness to the human society

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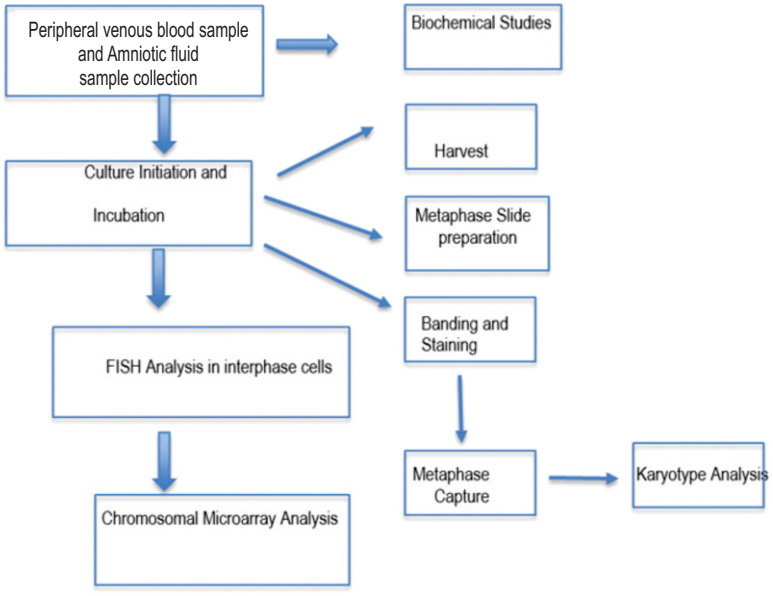
Abstract

Aim: 49, XXXXY syndrome is a rare chromosomal abnormality with an approximate incidence of 1:85000 – 1:100000. Early diagnosis is important to improve the quality of life of patient. This study was carried out to assess the chromosomal abberations in prenatal and postnatal sample of patients through cytogenetics study.

Methodology: Sex chromosomal aneuplodies were performed in both amniotic fluid and peripheral venous blood samples. Cytogenetic studies such as Karyotyping was done in in-vitro culture cells by GTG banded metaphase slides. FISH test was conducted in raw sample to rule out low grade mosaicism and DNA isolated from both the samples to rule out the gain and loss of genes by Chromosomal microarray technique.



Results: The two cases performed showed aneuploidy with pentasomy sex chromosomes in Karyotyping in all the metaphase analysed and FISH test resulted with five signals in sex chromosome with four copies of X chromosomes and one copy of Y chromosome in all the cells. There was no loss of genes and only gain of genes in X chromosomes as identified in Chromosomal microarray.

Interpretation: Based on the findings, antenatal screening is essential and should not be avoided for any USG findings and biochemical screening. The test should not be limited only to trisomy studies. Extensive studies should be performed to rule out the genetic disorders. Chromosomal aneuploidies can be identified by cytogenetic studies like Karyotyping , FISH and Chromosomal microarray technique.

Key words: Aneuploidy, Chromosomal microarray analysis, Cytogenetics, Fraccaro syndrome, Karyotyping, Pentasomy sex chromosome

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Introduction

Sex chromosomal abnormalities are mostly identified with aneuploidies. Patients with pentasomy sex chromosomes will have some pecuiliar phenotype with dysmorphic features, reproductive organs with ambiguous genetalia,delay motor development and cardiac defects. Sex chromosomal aneuploidies are one of the most common chromosomal disorders seen in (1:500) live births. Sex of a human is determined by X/Y chromosomes. Male sex chromosomes is typically with one X and one Y chromosome whereas female is typically with two X chromosomes. Sex chromosomal aneuploidy with single X chromosomes, known as Turner syndrome, is seen in females. It is a developmental sexual disorder with X and Y chromosomes with female phenotype with a additional sex chromosomes like 47,XXY; 47,XYY; 48,XXXX; 48,XXYY and 49,XXXXY (Thompson *et al.,* 2022). The most common cause or reason for sex chromosomal aneuploidy is non disjunction which occurs in post zygotic development or meiosis. The frequency of diagnosing sex chromosomal aneuploidies is less compared to autosomal aneuploidies due to less severity of phenotypic changes. Pentasomy sex chromosome is a rare sex chromosome and only very few cases have been reported so far. It was first reported by Fraccaro and his co-workers (Fraccaro *et al.,* 1960), therefore, it is also termed as Fraccaro syndrome. It is an unusual aneuploidy seen with pentasomy sex chromosome. It is one of the rare aneuploidy found in 85000-100000 males. (Gropman *et al.,* 2010).

Numerical chromosomal abnormalities in sex chromosomes are defined as sex chromosomal aneuploidies. Developmental dyspraxia is the main cause of language and motor impairment (Burgemeister *et al.,* 2019). 49,XXXXY syndrome is usually not inherited and caused by a random error in cell division. Males with more than one extra sex chromosome are termed as Klinefelter syndrome or Klinefelter variants.The symptoms are not common and have unique physical and behavioral features. 49,XXXXY syndrome patients have a distinct appearance, including characteristic facial features like deep seated eyes, flat depressed nasal bridge, dysplastic ears, cardiac defects, multiple skeletal anomalies, clubbed feet, abnormalities in the genitalia, mental deficiency, and speech problems, which are the unique characteristic features other than Klinefelter syndrome patients (Etemadi *et al.,* 2015). Chromosomal anomalies have been reported to contribute as one of the crucial genetic factors in male infertility. In this study, two case studies, antenatal and postnatal cases, are reported where molecular studies revealed pure 49,XXXXY syndrome in South Indian patients.

Materials and Methods

a) 1st case – Postnatal: A 2-year-old boy born to a 23- year- old mother in her first pregnancy by C-Section. No complications were identified during antenatal periods and no genetic testing was conducted. The parents had three degrees of consanguinity, and the pregnancy was uncomplicated. No other family member was identified with chromosomal abnormality.

Physical examination of the child revealed vitally active, well-developed and well-nourished with delayed oral motor dyspraxia and mental retardation. Due to motor developmental and speech delay, he was taken for a neonatal check-up to AKG Memorial Co-operative Hospital, Kannur, Kerala. The body weighed 17.7 kg, 93cm tall, and 31cm cephalic circumstance. Upon physical examination, the patient was identified with a slightly flat depressed nasal bridge and microcephalic head. Ears and eyes were normal, and pupils were equal and reactive. No other defects like skeletal defects, craniofacial anomalies, cleft palate, hypogonadism, diabetics or hypothyroidism were identified. External genitalia was checked for micropenis and undescended testes were normal. Central nervous examination of the heart was 80/ min with sinus rhythm and no murmuring was observed. Abdominal ultrasound for kidney, ureter and urinary bladder was normal. Chest X-ray was normal and Echocardiography showed an Atrial septal defect with moderate size. Angiography revealed blood flow from left to right shunt to be 5 mm. Biochemical test parameters were within the normal range, except Testosterone, Triglycerides and Vitamin D (Table. 1). The patient was supplemented with Norma brain -800 mg. To further confirm the genetic factor, a pheripheral blood sample was collected and refered for a genetic test in Life Cell International Pvt. Ltd., Chennai. Karyotyping was performed in GTG banded slide. Fluorescence *in-situ* hybridization (FISH) analysis was performed in interphase cells using probes for chromosomes X and Y, and CMA analysis was performed in isolated DNA sample.

b) 2nd Case – Antenatal: A 25-year- old patient with 16 weeks 3 days of gestational period went for a consultation to a Maternal Care Center, Kerala. She underwent an ultrasound as a regular second-trimester checkup. The ultrasound report showed increased nuchal translucency, which was 2.6mm in size and also

Table 1: Biochemical tests

Parameters	Results	Normal Range
T3	214	105-245 ng dl^{-1}
T4	8.5	7.8-16.5 ug dl^{-1}
TSH	2.78	0.4-10 ul U ml^{-1}
Serum Cholesterol	172	140-200 mg dl^{-1}
Calcium	10.40	9.0-11.0 mg dl^{-1}
Phosphorus	6.01 mg dl^{-1}	4-7 mg dl^{-1}
Alkaline Phosphates	239	60- 321 U l^{-1}
Testosterone	< 1.0 ng ml^{-1}	2.27-9.76 ng ml^{-1}
Vitamin D	19.84	30-100 ng ml^{-1}
Triglycerides (2)	500	<161 mg dl^{-1}
Bilirubin Total	0.3	0.22-1.0 mg%
Serum Protein	7.7	6.2 – 8.0 gm%
Serum Albumin	4.3	3.5-5.5 gm dl^{-1}
Globulin	3.4	2.0-3.5 gm dl^{-1}
SGOT	33	0-35 U l^{-1}
SGPT	14	0-45 U l^{-1}

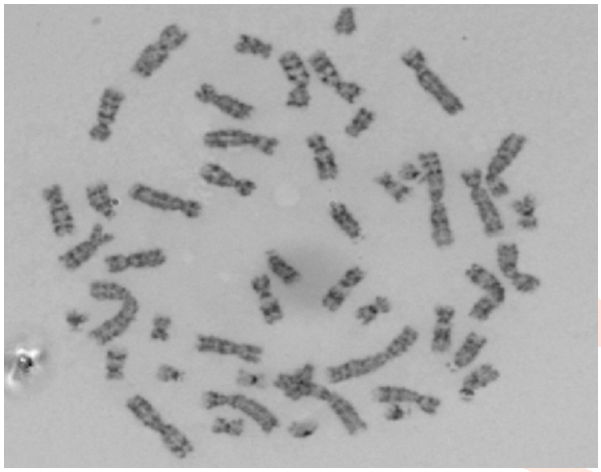

Fig. 1: Metaphase spread of 49,XXXXY observed under inverted phase contrast microscope.

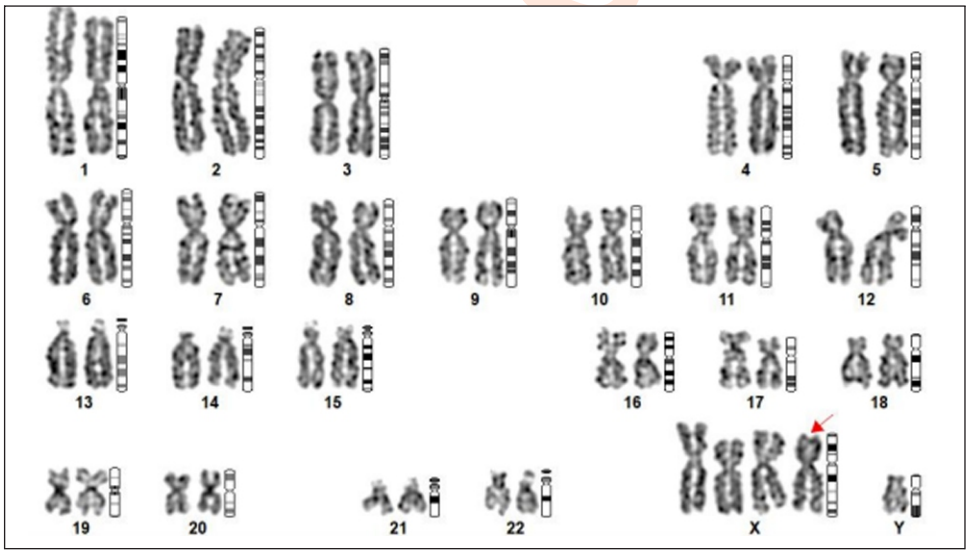

Fig. 2: Karyotype of 49,XXXXY analysis done in ASI software shows the presence of three extra copies of X chromosome in male patient.

with unossified nasal bone. Combined other biochemical test showed a high risk for Trisomy 21 (Down's syndrome).

For further confirmation, the amniotic fluid sample was collected with patient consent for genetic testing. Genetic tests were conducted in Life Cell International Pvt. Ltd., Chennai. Amniotic fluid sample was processed and inoculated in 25 cm2 culture flask and incubated in 5% CO2 supplemented incubator. Once it reached confluency, the cells were harvested, metaphase spread slides were prepared and karyotyping was performed in GTG banded slide. FISH analysis was performed in interphase cells using probes for chromosomes X and Y, and CMA analysis in the raw sample.

Results and Discussion

The postnatal and prenatal samples performed for Karyotype on 50 metaphases in GTG banded metaphases revealed a total number of 49 chromosomes, with 3 extra X chromosome copies in all metaphases (Fig. 1). FISH analysis of 200 cellular interphase and metaphase for 20 cells using probes for chromosomes X and Y, indicated 100% for 49,XXXXY (Fig. 2). CMA analysis showed a duplication spanning 155,065kbp (~155MB) on chromosome X. The duplication had four copies of X chromosome (Fig. 3). Aneuploidy with 49, XXXXY karyotype occurs during non-disjunction of the maternal X chromosome

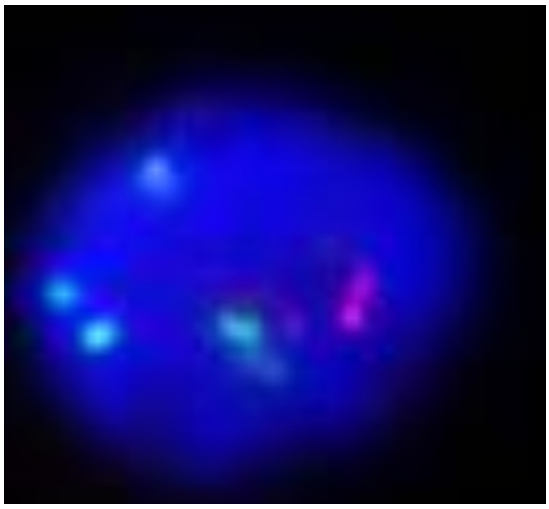

Fig. 3: Flurosecence *in situ* hybridization for 49,XXXXY with four green signals (X) and one red signal (Y).

during cell division from both meiosis I and meiosis II. Nondisjunction is the malfunction of homologous chromosomes or sister chromatids that fail to separate properly during Anaphase 1, which results in the development of a secondary oocyte with extra copies of X chromosomes, if it gets fertilized by a Y-bearing sperm, the embryo will result with 49, XXXXY syndrome (Patacchiola *et al.,* 2012). Previously it was identified as the variant in Klientfilter syndrome, later it was identified with its distinct characteristic features. 49, XXXXY syndrome has a different appearance with unique features like genital malformations such as hypospadias, hypogonadism and cryptorchidism. They also have a coarse face, hypertelorism, epicanthic folds, neurocephalic, and upslanting of palpebral fissures. Skeletal defects are radioulnar synostosis and microcephaly (Hammami et al., 2020; Buller et al., 2018).

Adults affected by 49, XXXXY syndrome are short stature, unlike those identified with Klinefelter syndrome. Various reports have classified it as "the classic triad" of radio-ulnar synostosis, hypogonadism and mental retardation (Naotunna *et al.,* 2021). Usually, persons with 49, XXXXY syndrome are identified with intellectual disability and developmental delay. Dyspraxia is a developmental coordination disorder that is the main cause of language and developmental motor impairment (Burgemeister *et al.,* 2019). Mental retardation is one of the major problem in 49,XXXXY patients. A direct correlation determined between the extra copies of supernumerary X chromosomes with phenotypic appearance and mental retardation has been well reported. The severity of mental retardation increases with each additional X chromosome (Etemadi *et al.,* 2015). Our 2-year-old patient was also identified with mental retardation, due to increase in the number of X chromosomes. Hypotonia or poor muscle tone leads to developmental delays in motor skills. Our 2-year-old patient, showed developmental delays, such as sitting, standing and walking were identified and documented. He was unable to walk, sit or stand without support till 2 years of age, with delay in speech and inability to hold objects with his hands. In Klinefelter syndrome patients, congenital heart defects are identified nearly 15% to 20% (Salzano *et al.,* 2016). Most patients with 49,XXXXY syndrome are also identified with congenital heart defects like Fallot's Tetralogy, Atrial septal defect (ASD), Patent ductus arteriosus (PDA), Ventricular septal defect (VSD), and pulmonary stenosis (Araimi *et al.,* 2020). Our patient was also identified with ASD with pulmonary hypertension. Prenatal detection of 49,XXXXY is unusual and may be incidental due to non-specific ultrasound findings. Antenatally, some fetuses may be identified with some abnormal findings in USG like a flat depressed nasal bridge, unossified nasal bone, increased NT, intrauterine growth restriction, and also low birth weight in post-

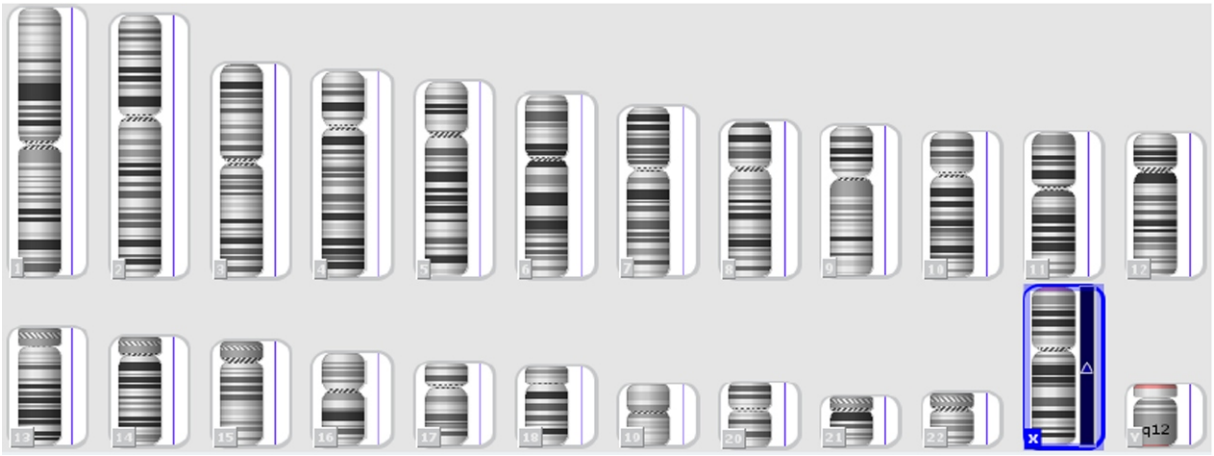

Fig. 4: Chromosomal microarray describes the spanning of tetra X chromosomes

delivery (Wei *et al.,* 2019). In our antenatal case, USG showed increased NT (2.6mm) with unossified nasal bone and the Combined test shows an increased risk for trisomy 21 (1:192) but the fetus was identified with 49,XXXXY syndrome in chromosomal studies.

The frequency of diagnosis is less in sex chromosomal aneuploidy compared to autosomal aneuplodies due to less sevearity of phenotype in the affected patients.Few sex chromosomal aneuploidies are diagonized in females during the pubertal stage.49, XXXXY syndrome is a rare chromosomal abnormality with an approximate incidence of 1:85000 – 1:100000. In LifeCell Laboratory, in the last three years, only two cases have been identified with 49,XXXXY syndrome. Early diagnosis is important to improve the patient's quality of life. Based on our findings, antenatal screening is essential and should not be avoided for any USG findings and biochemical screening. The test should not be limited only with trisomy studies. Extensive studies should be performed to find out the genetic disorders. This kind of chromosomal aneuploidies can be identified by Cytogenetic studies like karyotyping ,FISH and Chromosomal microarray.

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Conflict of interest: The authors declare that there is no conflict of interest.

Data availability: Karyotype and FISH images available as backup storage and DNA available.

Consent to publish: All authors agree to publish the paper in *Journal of Environmental Biology.*

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