



FRAGILE X SYNDROME: CLINICAL & CYTOLOGICAL STUDY IN NORTH KARNATAKA

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ABSTRACT

Fragile-X syndrome (FXS) is the most common inherited form of mental disorder. The clinical features of FXS include moderate to severe mental retardation, dysmorphic facial features. One such case was seen & admitted in our hospital with similar clinical symptoms. We examined both clinical and cytological analysis. After examining all these we found child with Fragile X Syndrome. We suggest that molecular analysis of Fragile X Syndrome related to FMR1 gene will help to know the novel mutation in this population, which will be helpful for early diagnosis of Fragile-X syndrome and type of genetic disorder.

Key words: *Fragile X Syndrome (FXS), Mental retardation, Cytogenetic Analysis, Clinical case Fragile X Syndrome.*



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INTRODUCTION

Fragile X Syndrome (FXS) is inherited form of mental retardation which will affect around one in 4,000 males and one in 8,000 females¹. Incidence population is still unknown although its prevalence is known to be high. Recent studies have shown a pre-mutation prevalence in men (1:430) and women (1:209) in the USA² The clinical features of FXS include moderate to severe mental retardation, dysmorphic facial features (macrocephaly, long face, prominent ears, high-arched palate), joint laxity, post pubertal macro-orchidism and behavioral anomalies (hyperactivity, aggression, timidity, autistic traits). Seizures are reported to occur in 10 to 20 percent of cases with a full mutation FXS¹. It is a complex type³. Primary features of Fragile X Syndrome are mild to severe mental retardation (MR), macro-orchidism⁴ associated with connective tissue disorder such as hyper extensible finger, double jointed thumbs, etc. In most cases, a fragile site is detected at Xq27.3, which harbors the FMR1 gene⁵. This is caused by mutation of a gene located at q27.3 of the long arm of X chromosome. CGG nucleotide sequences are increased with number of repetitions. A healthy patient might exhibit 6-54 repetitions, FXS patients' exhibit 1,000 to 2,000⁶. The cognitive, behavioral and physical phenotypes of this syndrome vary by sex, with males

being more severely affected because of the X-linked inheritance of the mutation.

Case Report

A 10 year old male child with partial inability to speak & delayed attainment of milestones, also with NICU admission (on day 2 of life) for fever. His father is mentally retarded & his 2 male siblings died on day 4 & day 6 of life.

Clinical Examination

Child had microcephaly, poor eye contact, bilateral telangiectasia, convergent squint, beaked appearance of nose, prominent ear, hand flapping, prominent right ear, hand flapping, hand biting, a large cafe au lait spot (2x2 cm) on right side of umbilicus & flat feet. MRI of Brain, and diffusion weighted imaging & ADC mapping done. The results showed global paucity of white matter with thinning of corpus callosum, mild cerebral atrophy. Thus suggests the cause as possibility sequelae of hypoxic ischemic encephalopathy. Cytological Analysis was done to see the chromosomal structural changes. We also saw long narrow face & prominent ears as compared to normal cases, as shown figure 1. We also observed child is facing to open the eyes (eye lid movement) as shown in figure 2.



Figure 1 & 2
FXS with long narrow face, prominent ears shown, poor eye contact

Cytological Examination

A 1 ml of peripheral heparinized blood was taken from patient and then immediately transferred to Laboratory of Genetics. According to standard procedures was performed on the fragile X syndrome Karyotype⁷. In this study, the RPMI 1640 medium containing 25% fetal bovine serum (FBS), antibiotics such as Penicillin (300 mg/ml) and thymidine (300 µg/ml) were used (products of GIBCO and SIGMA). Medium under the Laminar Air Flow, were prepared. For cultivation, 5 ml of cell culture medium in each tube, 0.1 ml Phytohemagglutinin (PHA) and 0/5 ml peripheral blood were added and incubated for 72 h with 5% CO₂ at 37 ° C respectively. Tubes containing medium every day were gently shaken. After this, 0.1 ml of colcemid was added to each tube and

after half an hour harvesting steps were done. Tubes were placed for 15 minutes in the serologic bath. After centrifugation, at 1200 rpm for 10 min, cells isolated from the culture medium were impressed with the hypotonic solution (KCl; 0/75 M). After centrifugation, the cells were exposed to the fixative solution (acetic acid and methanol at a ratio of 1 to 3) and they were centrifuged again. After several washing steps with fixative solution, a clear suspension of lymphocytes obtained. Drop shot technique was used with sterile Pasteur pipette several slides were prepared.⁸ With the G- banding method, metaphase spreads were prepared on the slides⁹. First, metaphase spreads were exposed to trypsin for 15 seconds then placed in Giemsa solution. After 10 minutes, the slides were washed with distilled water. Pictures were taken from slides of

each patient and with karyotyping software, they were analyzed and descriptive statistics were diagnosed. Cytological Analysis revealed that, in total 46 chromosomes 20 were metaphase, total 44 were autosomes these were observed under 350 band

resolutions. Out of 46 chromosome 45 chromosome were having telomeric part were missing (45 X) as shown in figure 3, this is unique feature of fragile X Syndrome cases

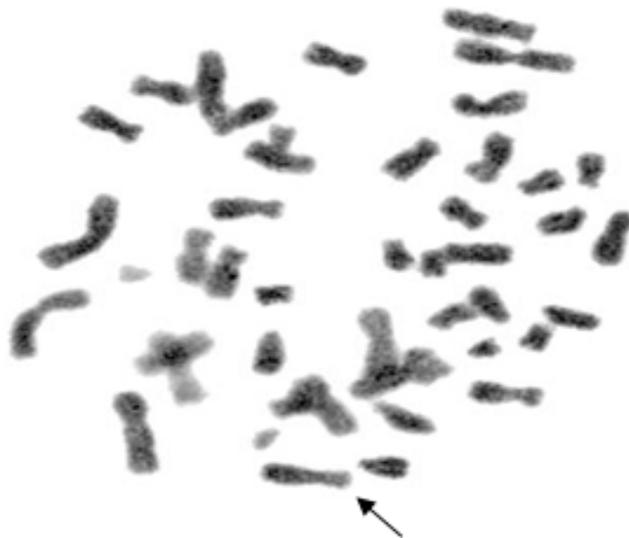


Figure 3
Chromosomes were obtained by cytogenetic method arrow mark shows some telomeric part is missing.

DISCUSSION

The fragile X syndrome is a genetic disease with a great variability in clinical presentation. Until the 1990s, this syndrome was diagnosed by clinical signs and chromosomal study (karyotype). Recent studies show that PCR method is not sufficient evidence to detect mutation for women detected by disease. Clinical & molecular evidence helps to diagnose FXS Syndrome. Fragile X Syndrome (FXS) is a common inherited form of mental retardation which will affect around one in 4,000 males and one in 8,000 females¹. This syndrome is having a dominant X-linked trait with incomplete penetrance (80% males, 20% females). It is caused by an amplification of the repeated CGG in the non-transcribed region towards 5' of the FMR1 gene promoter located at the FRAXA locus of the chromosome X in Xq27.3⁵. Due to the FMR1 gene permutation and FMRP expansions, variable effects have been observed in the phenotypic constitution of syndromic patients^{10,11}. In our study, we observed that both clinical & cytological examination for the FXS Syndrome was this patient having all the clinical symptoms & cytological evidence for the syndrome. Other studies reported classic facial characteristics in individuals with FXS, such as long face, downward mandibular rotation, and skeletal open bite¹² do not meet our results regarding the increase in the mandibular angle. Our results suggest that the long face observed in the patients with FXS¹³. At least three possible explanations could be given for the observed discrepancy between clinical and genotypic

assessments. Firstly, clinical features described as characteristic for FXS may not be evident in all children having the relevant mutation for FXS. The reason is that the age of appearance of FXS phenotypic features and their degree of expression vary among individuals¹⁴. A longitudinal clinical investigation revealed that although genetically positive, the phenotypic features change with age and are most prominent only by 10 to 15 years¹⁴.

CONCLUSION

We suggest that molecular analysis of Fragile X Syndrome related to the FMR1 gene will help to know the novel mutation in this population, which will be helpful for early diagnosis of Fragile X Syndrome type of genetic disorder. It will be helpful to practitioners to diagnose and treat the disease which will in turn help to our society.

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CONFLICT OF INTEREST

Conflict of interest declared none.

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