IJBPAS, September, 2015, 4(9), Special Issue: 82-92

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

THE PREVALENCE OF FRAGILE X SYNDROME AMONG PEOPLE WITH MENTAL RETARDATION FROMEAST AZERBAIJAN PROVINCE

PEYMAN HADI[1], FATEMEH KESHAVARZI[2*]

1-Department of biology, Kurdistan Science and Research Branch, Islamic Azad University, Sanandaj, Iran

*****2-Department of Biology, Sanandaj Branch, Islamic Azad University, Sanandaj, Iran

***Corresponding Author: E Mail: gol.keshavarzi@gmail.com; 09183704918**

ABSTRACT

Fragile X syndrome is the most common cause of inherited mental retardation. Patients with fragile X syndrome show variable mental disability, typical long and narrow facial appearance with large ears and prominent fontanelle and frequent macro-orchidism. The fragile site is on the long arm of X chromosome in X q27.3 region. Incidence of syndrome is 1 in 2000 in males and 1 in 2500 in females. This fragile site is visible only with using of special cultural technices. At the molecular level, the fragile X syndrome is associated with an amplification of CGG repeat sequence of the FMR1 gene. The aim of this study was to determine FXS prevalence in moderate Mentally retarded people in East Azerbaijan province.

Fifty Person with moderate mental retardation who were clinically suspicious to have FXS were screened for fragile X chromosome by using cytogenetic and molecular methods. Blood samples were collected and cultured in specific culture media. G-Banding method was used for karyotyping. To ensure correct results of cytogenetic testing, Four suspected case of fragile X syndrome were tested by pcr. Results were analyzed using logistic regression.

Fifty patients were in the category of mild to moderate mental retardation. 4 patients (8%) were found to express fragile X site at q27.3. The results showed that the fragile X syndrome and familial relationships, economic status, place of residence, there is no significant association, but the fragile X syndrome and mental retardation in the family history was significant.

The frequency of fragile X positive cases found in this study is similar to other reports of fragile X syndrome in preselected patients.

Keywords: Fragile X syndrome, FMR1, karyotype, mental retardation

INTRODUCTION

Fragile X syndrome (FraX) is one of the most prevalentgenetic causes of developmental disability, representing the most frequent form of inherited severe cognitive deficit, second only to Down syndrome as a genetic cause of mental retardation. It is estimated that the FraX affects approximately 1 in 2,500 individuals [1-4].The syndrome is inherited as an X-linked dominant trait with reduced penetrance: 80% in males and 30% in females[5]. According to studies conducted in Iran The frequency of fragile X syndrome 63% have been reported [6]. there is no statistical analysis concerning this field in Azerbaijan.

The syndrome is mainly characterized by a variable degree of mental retardation,typical long and narrow facial appearance with large ears,prominentfontanelle and large testes[7]FXS can be cytogeneticallydiagnosed by the expression of chromosome X-fragile site atband Xq27.3.[8] The unique mutation that results in FXSconsists of expansion of the CGG trinucleotide repeats (>200)in the 3' untranslated region of the FMR-I gene at Xq27.3 aswell as hypermethylation of the repeat and its flanking regionresulting in absence of the FMRI protein.[9]

FMR1 is a highly conserved gene that consists of 17 exons and spans ~38 kb (10-11). Within the 4.4 kb of *FMR1* transcript, a CGG trinucleotide repeat is located at the 5′-untranslated region (5′-UTR). Among normal individuals, this CGG repeat is highly polymorphic in length and content, often punctuated by AGG interruptions [12,13,14,15,16]. The normal repeat size ranges from 7 to ~60, with 30 repeats found on the most common allele. In most affected individuals,CGG repeats are massively expanded over 230 repeats (full mutation) and becomes abnormally hypermethylated, which results in the silence of the *FMR1* gene. Alleles with between 60 and 230 CGG repeats are called premutation. They are generally unmethylated with normal transcript and protein level, but are extremely unstable during transmission to next generation[17-18]. Expansion of premutation into full mutation can only occur by maternal transmission and depends on the length of the maternal pre mutation. Due to X-linkage,

affected males have more severe phenotypes than affected females, whose phenotype is modulated by the activation ratio of the normal X chromosome. Identification of other mutations of the *FMR1* gene, such as deletions and pointmutation among patients with usual phenotype but without fragile site expression, firmly established that the *FMR1* gene is the only gene involved in the pathogenesis of fragile X syndrome [19-20]. Thus, the absence of the *FMR1* gene product, fragile X mental retardation protein (FMRP), is the typical cause of fragile X syndrome.[21]

Laboratory diagnosis of fragile X syndrome is done by cytogenetic studiesOr by molecular methods such as PCR and Southern blot analysis.The fragile site on the long arm of X chromosome in X q27.3 region Observedin10% to 40% of cells. This study was to evaluate the usefulness of the Cytogenetic tests prior to undertaking molecular tests for the diagnosis of patients with FXS as a trial to reduce the laboratory load Diagnosis of fragile X syndrome by cytogenetic studies

5% chance of error detection Which can be solved using molecular methods ;[22-23-24] thus To ensure correct results of cytogenetic testing,7 suspected case of fragile X syndrome That was possible Show a false positive results were tested by pcr.

MATERIALS AND METHODS

This study included 50 mentally retarded (MR) males. They were selected from mental retardation center of East Azerbaijan province.

Cytogenetic methods:

For routine cytogenetic analysis, 0.3 mL of peripheral blood were incubated in complete lymphocyte culture medium (10% fetal bovine serum in RPMI 1640, with 0.15% phytohemagglutinin and 1% penstrept) in 5% CO2 incubator at 37°C for three days. Metaphases were harvested by adding colcemid for 20 minutes, followed by hypotonic KCl treatment for 5 minutes and fixation using standard 3:1 methanol-acetic fixative (all the reagents were from sigma).

High-resolution study was done by synchronization using methotrexate (10-7 M) for 17 hours and thymidine (10-5 M) for 5.5 hours before harvesting, as described elsewhere [25]. For fragile X study, we examined two sets of cultures: a low-folate medium (M 199 culture medium), and a methotrexate-containing medium for the last 24 hours of culture[26].4 Cases were considered positive for fragile X if 5% or more of the examined 100 cells showed the characteristic fragile site. In all studies,

microscopic examination of 20 cells and photography of two cells was done after standard Trypsin- Wright G-banding (GTW) and/or Quinacrine Q-banding (QFQ). Chromosomes were visually analyzed, and abnormalities were detected during microscopy in all cases.

MOLECULAR METHODS

Genomic DNA from peripheral blood lymphocytes by standard method of salting was extracted. Primers for Amplification of FMR-1 Gene, designed and produced [27]. Amplification products were resolved by %8 Polyacrylamide gel electrophoresis (PAGE).The gels were silver-stained according to bassams protocol[28]. Molecular analysis (PCR) was done, and to identify and confirm the repetition of three nucleotides (CGG) were analyzed by Southern blot. Genomic DNA by the restriction enzyme HindIII and methylation-sensitive restriction enzymes EclXI. DNA samples were digested, were size-separated by electrophoresis on a % 0/8 agarose gel with using a DIG- labled molecular marker and transferred to a positively charged nylon membrane. The stbprob specific for fragments containing the CGG repeat was labeled using a non-radioactive label. After hybridization, the membrane was washed and labeled probe was detected by exposure to an X-ray film.

RESULTS

In this study 50 blood samples were taken from the male patients. The distribution of the mental retardation males according to the age at diagnosis is shown in Table (1) and studied eight fragile X-related features in the mental retardation males shown in table (2). Nineteen cases (56%) were diagnosed after the age of 10 year. Thekaryotypic results were showed among Numbers, %8 (4 cases) have fragile x syndrome (fig.1 to 3). In 50 patients studied, parental consanguinity was found in 2 patients (4%), and family history of mental retardation was foundin14 cases (28%) also 43families (86%) were poor economic condition. The results of the chi-square test showed that the cases cited above, Only the Fragile X syndrome and a history of retardation, there was a significant association.

Table (1): Distribution of the mental retardation males According to the Age at Diagnosis

Age	No. of patients	%
≤ 5 y	4	8
> 5-10 y	17	34
> 10-15 y	19	38
> 15-20 y	10	20
Total	50	100

Table (2): Fragile X Features in the mental retardation males

Fragile X-related features	Not seen	Minor	Medium	Severe
Long face	22	5	9	14
Large prominent ears	30	7	4	9
Hypre-extensible joints	21	11	5	13
Macro-orchidism	44	0	0	6
Hyperactivity	6	9	12	23
Autistic features	13	7	11	19
Biting the hand	34	3	4	9
Unusual speech pattern	10	17	9	14

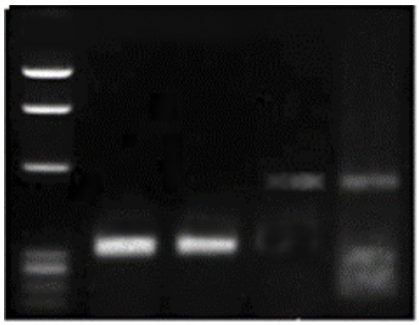


Fig1. Line 1 (left) is a known marker; Line 2 and 3 are control. Line 4 and 5Are patients

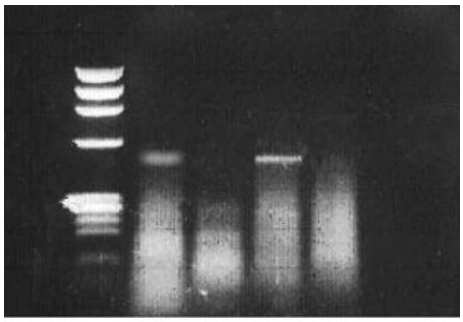
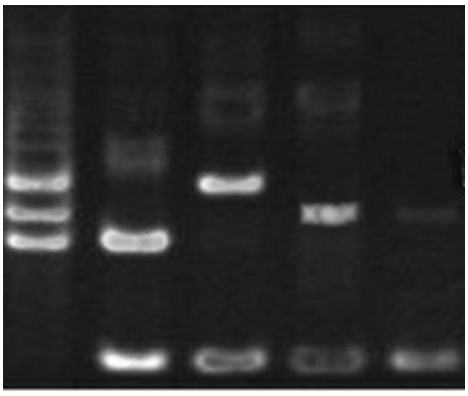


Fig 2. First line (left) serve as a known; Marker. Line 1 and 3 has normal alleles; Line 2 and 4 has FGX.



M 1 2 3 4

Fig 3: There FXS in line 4. In line 1 to 3; Represent normal alleles

DISCUSSION

Fragile X syndrome is the most prevalent form of familial mental retardation in the world. Therefore analysis and recognition the carriers of the syndrome has a considerable importance all around the world. Since there is no statistical analysis concerning this field in Azerbaijan, thus collected data on incidence of the disease in the district, has a valuable importance in management of the disease, genetic consultation, and designing future plans for patients. The study was aimed to determine the linkage between Fragile X syndrome and mental retardation in some of the patients maintained in "center of disabled people of east Azerbaijan". According to the results of the study which was done on 51 case of mental retardation in east Azerbaijan, 8% of them had Fragile X syndrome. Past studies on the prevalence of fragile X syndrome, based on cytogenetic diagnosis has been made, 0.4-0.8 of the 1000 for men and 0.2-0.6 of 1000 for women, was reported. Studies based on molecular methods, the frequency of fragile X syndrome in European countries, the US and Australia have shown that is 0.6 per thousand [29].

According to studies performed in Turkey (which has a similar race to Azerbaijan) 14 of 120 mental retardation case had Fragile X syndrome; However its frequency in Turkey is 11.7%[30]. Another study performed in Antalya province of Turkey, showed that 17 of 132 patients had Fragile X syndrome which its frequency is 12.87%[31].

Jenkins reported the frequency of this syndrome 6.3% among mental retardation cases and Butler reported 6.4% as the frequency [32-33].In selected populations of mentally retarded patients, an overall prevalence rate of 4.8% of fragile X was reported by Proops et al [29].Carpenter et al; studied 36 patients with a family history of MR and found 13.9% to be fragile X-positive cases. Iqbal et al; studied 81 patients with a family history of MR; among these, 12 patients (14.8%) were found to be fragile X-positive, which is similar to the report by Carpenter et al[34-35].Froster reported only 3.6% as the frequency in a similar Study which was done on 200 mental retardation case with positive familial history of the disease[29]. In our study, 3 of 4 cases of fragile X syndrome patients had familial history of mental retardation.

In studies performed in various countries, there are some specific phenotypes defined for patients with fragile X syndrome, which are the primary criteria to diagnose the patients. Our study in Azerbaijan showed that most of men suffering fragile X syndrome

had large testis, large ears, long faces, attention disorders and weak eye contact.

There is no cure for fragile X syndrome until now, although appropriate decisions and drugs can improve the ability of individuals [36]. The increased understanding of the molecular mechanisms of disease in FXS has led to the development of therapies targeting the affected pathways. Evidence from mouse models shows that mGluR5 antagonists (blockers) can rescue dendritic spine abnormalities and seizures, as well as cognitive and behavioral problems, and may show promise in the treatment of FXS.[37][38][39] Two new drugs, AFQ-056 (mavoglurant) and dipraglurant, as well as the repurposed drug fenobam are currently undergoing human trials for the treatment of FXS.[41][40] There is also early evidence for the efficacy of arbaclofen, a GABAB agonist, in improving social withdrawal in individuals with FXS and ASD [37][11].

Management of FXS may include speech therapy, behavioral therapy, sensory integration occupational therapy, special education, or individualized educational plans, and, when necessary, treatment of physical abnormalities. Persons with fragile X syndrome in their family histories are advised to seek genetic counseling to assess the likelihood of having children who are affected, and how severe any impairments may be in affected descendants.[42]

Given that one of the main causes of mental retardation in the world is fragile X syndrome Thus, with proper planning and delivery mechanisms and genetic counseling can be a step towards reducing the birth of such people in society that All of the above are required to inform the incidence of this syndrome in the region, which In this study, the frequency was determined in Azerbaijan.

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