A survey of patients with mental retardation of unknown origin

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Abstract

Introduction: Fragile X syndrome (FXS) is one of the most prevalent genetic causes of developmental disability, representing the most frequent form of inherited severe cognitive deficit. The present study was undertaken to investigate FXS and its prevalence in moderate mentally retarded people in patients.
Materials and methods: Nineteen people with moderate mental retardation (MR) who were clinically suspicious to have FXS were screened for FXS 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 FXS were tested by PCR. Results were analyzed using logistic regression analysis.
Results: Four patients (4%) were found to express fragile X site at q27.3. The results showed that the relationship of FXS with familial, economic status was not significant, but the relationship of FXS with MR and family history was significant.
Conclusion: The frequency of FXS positive cases found in this study is similar to other reports of FXS in preselected patients.

Keywords: MR, FXS, FMR1, Cytogenetic

Introduction

Mental retardation (MR) means delay in mental development; it means an impairment of the intellectual processes of the mind, making it difficult for the person to cope with environment in which they find themselves. FXS (FXS) is one of the most prevalent genetic causes of MR, representing the most frequent form of inherited severe cognitive deficit, second only to Down syndrome as a genetic cause of MR. It is estimated that the FXS 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 FXS have been reported to be 63% (6). The syndrome is mainly characterized by a variable degree of MR, typical long and narrow facial appearance with large ears, prominent fontanels and large testis (7). FXS can be cytogenetically diagnosed by the expression of chromosome X-fragile site at band Xq27.3 (8). The unique mutation that results in FXS consists of expansion of the CGG trinucleotide repeats (>200) in the 3' untranslated region of the FMR-I gene at Xq27.3 as well as hypermethylation of the repeat and its flanking region resulting 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

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normal individuals, this CGG repeat is highly polymorphic in length and content, often punctuated by AGG interruptions (12-16). The normal repeat size ranges from 7 to ~60, with 30 repeats found on the most common allele. In most affected individuals, a CGG repeat 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 permutation. They are generally unmethylated with normal transcript and protein level, but are extremely unstable during transmission to next generation (17). 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 point mutation 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 FXS (18). Thus, the absence of the FMR1 gene product, fragile X MR protein (FMRP), is the typical cause of FXS (18).

Laboratory diagnosis of FXS by various methods, such as Southern blot, PCR and Karyotype is detected and which in this study two methods, karyotype and PCR were used. Power to detect a suspected diagnosis of FXS karyotype is 99 percent for men and 95 percent for women have been reported (16-18). Due to high PCR accuracy for diagnosis of FXS, in 7 patients who showed signs of phenotypic Better, PCR analysis was used to identify these individuals. Since there is no statistical analysis concerning this field in east of Iran , 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.

Materials and methods

In this descriptive study, 90 mentally retarded (MR) males were selected from MR centers of Kermansh, Sanandaj and Abhar cities.

Cytogenetic method: 5ml of peripheral heparinized blood were taken from patients provided and then immediately transferred to Sanandaj Research Laboratory of Genetics and cell culture according to standard procedures was performed on the FXS Karyotype (11). In this study, the RPMI 1640 medium containing 25% fetal bovine serum (FBS) , antibiotics as Penicillin (300 mg/ml) and thymidine (300µg/ml) were used (products of GIBCO and Sigma). Medium under the hood and near the flame, were prepared. For cultivation 5 ml of cell culture medium to each tube, 0.1 ml Phytohemaglutinin (PHA) and 0.5 ml peripheral blood were added and incubated for 72 h with 5% CO_2 at 37 °C and incubated respectively. Tubes containing medium every day were gently shaken to the same medium. After this to each tube containing medium, 0.1 ml of colcemid was added and after half an hour harvesting steps was 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 and were impressed with the hypotonic solution (KCl0.75 M). After centrifugation the cells were exposed to the fixative solution (acetic acid and methanol at a ratio of 1 to 3) placed, and they were centrifuged again. After several washing steps with fixative solution, a clear suspension of lymphocytes obtained and drop shot technique was used with sterile Pasteur pipette several slides were prepared from each sample (12-13). With the G-banding method, metaphase spreads were prepared on the slides (14). First, metaphase spreads were exposed trypsin for 15 seconds then placed in Giemsa solution. After 10 minutes, the slides were washed with

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distilled water. Pictures taken from slides of each patient and using software karyotyping were analyzed and descriptive statistics were diagnosed.

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 (15). Amplification products were resolved by %8 Polyacrylamide gel electrophoresis (PAGE). The gels were silver-stained according to Bassam's protocol (16). Molecular analysis (PCR) was done, and to identify and confirm the repetition of three nucleotides (CGG) were analyzed by Southern blot. Genomic DNA digested by the restriction enzyme HindIII and methylation-sensitive restriction enzymes EclXI .

DNA that samples were digested and 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 stb probe 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

Among the patients studied, the karyotype results showed that 5 patients (6%) had FXS. Parental consanguinity was found in 4 patients (4.4 %), and family history of MR was found in 21 patients (23.3 %) also 56 families (62 %) had poor economic condition. Of the parameters evaluated in the study, only the history of retardation was significantly associated with FXS ($P<0.5$).

Discussion

The study was aimed to determine the linkage between FXS and MR in some of the patients with MR of unknown origin. According to the results of the study which was done on 90 cases with MR, 6% of them had FXS. Past studies on the prevalence of FXS, 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 FXS in European countries, the US and Australia have shown that is 0.6 per thousand (19). According to studies performed in Turkey (which has a similar race to Azerbaijan) 14 of 120 MR case had FXS; However its frequency in Turkey is 11.7% (20). Another study performed in Antalya province of Turkey, showed that 17 of 132 patients had FXS which its frequency is 12.87% (21). Jenkins (22) reported the frequency of this syndrome 6.3% among MR cases and Butler reported 6.4% as the frequency (23-24).In selected populations of mentally retarded patients, an overall prevalence rate of 4.8% of fragile X was reported by Proops et al (19). 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 (25).Foster reported only 3.6% as the frequency in a similar Study which was done on 200 MR case with positive familial history of the disease [19]. In this study, 3 of 4 cases of FXS patients had familial history of MR. In studies performed in various countries, there are some specific phenotypes defined for patients with FXS, which are the primary criteria to diagnose the patient. There is no cure for FXS until now, although appropriate decisions and drugs can improve the ability of individuals. The increased understanding of the molecular mechanisms of disease in FXS has led to the development of therapies targeting the affected pathways. 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 FXS in their family histories are advised to seek

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genetic counseling to assess the likelihood of having children who are affected, and how severe any impairments may be in affected descendants.

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