The prevalence of fragile X syndrome among people with mental retardation in Kermanshah Province
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ABSTRACT
Fragile X syndrome is the most common cause of inherited mental retardation in humans. People with varying degrees of mental retardation, long ears, forehead protruded, macroorchidism and obesity are identified. This syndrome is associated with a fracture of the long arm of the X chromosome (Xq27), that is visible in the spread of metaphase cells cultured in vitro with specific culture. The frequency of this syndrome in humans with different races, 1 in 1500 for men and 1 in 2500 for women have been reported. The frequency of this syndrome in Kermanshah Province was studied by preparing peripheral blood samples of 35 patients with mental retardation and culturing them in vitro. Karyotyping and molecular techniques (PCR) were used to detect the presence of fragile X syndrome. None of the patients had the fragile X syndrome.

KEY WORDS: fragile x, mental retardation, FMR1 gene

INTRODUCTION
Fragile X syndrome is the most common cause of mental retardation. The clinical features of this syndrome include long ears, forehead protruded, macroorchidism and obesity (Bulter, M.G., T. Hamill, 1995; Fengler, S., 2002; Mandel, J.L., 2004). Penetrance of this syndrome is 80% for men and 30% for women. The frequency of this syndrome is 1 in 1500 for men and 1 in 2500 for women have been reported. The penetration on the next generation increases, this particular case, increase in penetrance from one generation to the next, is the famous Sherman theory (Sherman, S., 1996). The frequency of this syndrome in humans with different races, 1 in 1500 for men and 1 in 2500 for women like have been reported in almost all human races (Anwar Iqbal, M., 2000; Burt, B.A., 1998).

Fragile X syndrome is associated with a fracture of the long arm of the X chromosome (Xq27), which is famous as the FRAXA site. This site is dedicated to the cultivation of lymphocytes with a broken point is visible. FMR1 gene as a cause of Syndrome X is known, is 38 kb in size and contains 17 exons, FMRP (fragile X mental retardation protein) protein product of this gene is linked to a number of RNA molecules include its encoded protein and acts as a carrier between the cytoplasm and the nucleus (Webb, T.P., 1986). At the beginning of exon 1, there are numbers of CGG repeats, in individuals with fragile X syndrome the number of iterations reaches 200 to 1000, leading to inactivation of the FMR1 gene (Burt, B.A., 1998; Webb, T.P., 1986; Hagerman, P.J., R.J. Hagerman, 2004). Laboratory diagnosis of fragile X syndrome by various methods, such as Southern blot, PCR and Karyotype is detected and which in this study two methods, karyotyping and PCR were used. Power to detect a suspected diagnosis of fragile X syndrome karyotype is 99 percent for men and 95 percent for women have been reported (Klauck, S.M., 1997; Penagarikano, O., 2004). Due to high PCR accuracy for diagnosis of fragile X syndrome, in 5 patients who showed signs of phenotypic better, PCR analysis was used to identify these individuals.

MATERIALS AND METHODS

2.1 patients:
In this descriptive study, 35 patients with mental retardation were studied in Kermanshah Province. After selecting patients, blood samples were taken from them.
2.2 Cytogenetic methods:

5 ml 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 were performed on the fragile X syndrome Karyotype (Jacobs, P.A., 1986). 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 Phytohemagglutinin (PHA) and 0/5 ml peripheral blood were added and incubated for 72 h with 5% CO2 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 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) 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 (Verma, R.S., A. Babu, 1995; Yunis, J.J., 1968).

With the G- banding method, metaphase spreads were prepared on the slides (Oosta, B.A., 1993). First, metaphase spreads Were exposed trypsin for 15 seconds then placed in Giemsa solution. After 10 minutes, the slides were washed with distilled water. Pictures taken from slides of each patient and using software karyotyping were analyzed and descriptive statistics were diagnosed.

![Fig. 1: Chromosomes were obtained cytogenetic method.](image1)

![Fig. 2: A) X-ray was taken from samples. B) PCR showing pictures in the gel.](image2)

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<th>Table 1: Morphological characteristics of persons with mental retardation.</th>
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2.3 molecular methods:

Genomic DNA from peripheral blood lymphocytes by standard method of salting were extracted. Primers for Amplification of FMR-1 Gene, designed and produced (Brown, W.T., 1993). Amplification products were resolved by 8% Polyacrylamide gel electrophoresis (PAGE). The gels were silver-stained according to Bassam’s protocol. 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 EcoXI.
DNA that samples were digested, and size-separated by electrophoresis on a % 0/8 agarose gel with using a DIG-labeled 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.

3. Results:
In this study, the 35 patients with cytogenetic methods was studied and preparation of karyotypes investigated. Images were taken from the karyotypes and analyzed by karyotyping software, and none of those cases were founded. There is no fracture in the long arm of the X chromosome (Xq27.3). Molecular study of 5 patients were suspected about fragile x syndrome(large testes) also showed that none of them did not show an increase in the repeat region (CGG) of FMR-1 gene.

4. Discussion:
The purpose of this study was to investigate the prevalence of fragile X syndrome among people with mental retardation in Kermanshah province. Based on the results, none of the patients were carrying the X Chromosome breakage (Xq27.3). Past studies on the prevalence of fragile X syndrome, based on cytogenetic diagnosis have been made, 0/4-0/8 of the thousand for men and 0/2-0/6 of thousand for women, were reported (De Viries, B.B.A., 1983; Jenkins, E.C., 1992; Turner, G.,1986). Studies based on molecular methods were showed the frequency of fragile X syndrome in European countries, the US and Australia have shown that is 0.6 per thousand (Froster-Icknies, U., 1983). In a study by Jacobs mental retardation were performed on three different populations and the frequency of fragile X syndrome in 1.9 % of men and 0.3% for women determined. In a study, which was conducted on a large population of mental retardation, the frequency obtained for FXS Was %4/8. In this study, over 35 persons with mental retardation that was in Kermanshah province, 30 peoples were from the village and 5 peoples were urban. Our results did not show fracture in the long arm of chromosome X (Xq27.3) that is different With the X syndrome results from other studies. In a study conducted by McGavran, showed that 7.5 % of mild mental retardation who had no information on the etiology of the disorder, they were FXS (McGavran, L., 1992). Carpenter et al, were examined 36 individuals with a history of mental retardation, and determined that 13.9 % of the individuals had fragile X syndrome (Carpenter, N.J., 1982). In a study of 81 individuals with mental retardation productivity with a family history of mental retardation in Saudi, the FXS, 41/8 % was reported.

However, our results were no cases of fragile X syndrome among people with mental retardation in Kermanshah province that show Is different with the results of other studies conducted in other parts of the world. According to the inheritance of this syndrome families who have a child with mental retardation are more chance to have children with fragile X syndrome and require screening tests like other countries were done (Proops, R., 1983).

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REFERENCES


