Frequency of fragile-x in x-linked mental retardation

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ABSTRACT

Introduction: Fragile X syndrome (FXS) is the most common form of inherited mental retardation and accounts for about one third of all cases of X linked mental retardation (XLMR). It is inherited as an X-linked dominant trait with a fragile site at Xq27.3 locus named fragile X mental retardation gene (FMR-1). The FMR-1 protein is widely expressed, with the highest expression in brain, testes, ovaries, esophagus, thymus, eye and spleen.

Patient and Methods: This study was conducted on twenty mentally retarded boys aged 8.5 ± 3.84 years, attending the genetic clinics at Menoufiya University hospitals. They represented 11 families.

All patients were subjected to detailed history, family pedigree, anthropometric measurements, thorough clinical examination with clinical scoring for the 13 items fragile X checklist, IQ assessment, routine investigations and cytogenetic studies which included conventional karyotyping using G banding and cytogenetic analysis for fragile X detection.

Positive consanguineous marriage was found in 15% of our studied cases. Nine families out of total eleven families had positive family history most of them were second degree relative males through maternal cousins.

Results: Craniofacial abnormalities included high arched palate in 65% of patients, large ears in 55%, prominent forehead in 45% and elongated face and abnormal teeth in 30% for each. Speech problems were present in 75% and hyperactivity in 55% of patients. Sixty five percent had mild mental retardation (IQ= 50-70%).

By applying the clinical scoring fragile X checklist, it was found that 3 patients (15%) had score more or equal to 19 and 3 (15%) had score from 16 to less than 19, while 14 (70%) had score less than 16.

As regards cytogenetic studies, 80% of our patients had normal karyotyping (46 XY) while four cases (20%) had positive fragile site on X-chromosome of whom two cases from the same family had 46, Y, Frg (X) (q27.3), while the other two cases, also from a single family, had inversion of Y chromosome beside positive fragile X chromosome site 46, Fra(X) (q27.3), inv (Y).

Conclusion: So, in a child with isolated mental retardation or autism of unknown etiology with considerable fragile X dysmorphic features or established family history of fragile X syndrome, chromosomal study that identifies the fragile site at Xq27.3 in addition to other cytogenetic abnormalities could be useful or early diagnosis and intervention by a special services team.

The present study revealed that the role of cytogenetic analysis in the diagnosis must be reevaluated since it can determine chromosomal abnormalities including the fragile X site with one single test, especially with unavailability of molecular techniques and their high costs.

A national multicenter genetic study of fragile X syndrome among affected children and their families is recommended to define our indications and steps of early diagnosis, population screening strategy, genetic counseling guidelines for different phenotypes and early intervention policies.

Key Words:

Fragile – X, cytogenetics, X linked mental retardation.

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INTRODUCTION

Fragile X syndrome is considered the most common known hereditary cause of X linked mental retardation affecting males and females in a X linked manner.[1]

X-linked mental retardation is a heterogenous group of conditions that have been classified as being syndromic (MRXS) and non syndromic (MRX) on the basis of their presenting symptoms.[2]

The most frequently mutated genes in X-linked mental retardation were methyl CPG binding protein, Aristaless related homeobox gene (ARX) and solute carrier family 6 member 8 (SLC6A8).[3]

The proteins of these genes were directly or indirectly involved in playing a role in the transmission of signals that regulate the development of neuronal axons and dendrites and in establishing and modulating synapses and regulating transcription, translation and fatty acid metabolism.[4]

The mutation of FMR1 gene located at Xq27.3, causing fragile X syndrome is due to expansion of CGG trinucleotide repeats within that gene.[5,6]

Fragile X syndrome is confirmed by cytogenetic diagnosis, Southern blot analysis, polymerase chain reaction (PCR), antibody based diagnostic test and linkage studies.[6]

The aim of this work was to find out the frequency of chromosomal abnormalities in a group of children with X-linked mental retardation with suggestive clinical features of fragile X-syndrome, to refine our early diagnosis to allow children to receive early intervention services and families to receive genetic counseling.

PATIENTS AND METHODS

The current study was conducted on twenty mentally retarded boys attending the genetic clinics at Menoufiya University hospitals, aged 2-16 years (8.50 ± 3.84). They represented 11 families.

Studied patients were selected as probable cases of fragile X syndrome since they had no history of perinatal insults,

no associated anomalies suggestive of other aberrations and their selection was based on 13 phenotypic features of the fragile X syndrome clinical checklist.[7]

These phenotypic features are mental retardation, hyperactivity, short attention span, tactile defectiveness, hand flapping, hand biting, poor eye contact, perserverative speech, hyperextensible metacarpopharyngeal joints, large or prominent ears, large testes, simian creases or Sydney lines and family history of mental retardation. Each item was scored 0, 1 or 2. Two points if the feature is present, one point if the feature was present in the past or is present to a borderline degree and zero point if the feature is absent.

Family pedigree was constructed, IQ was assessed using Wechsler-revised test[8], routine investigations were done and cytogenetic anlaysis for fragile X detection[9], and routine conventional karyotyping using G-banding were studied for all patients.[10]

RESULTS

The results of the present study were illustrated in Tables (1-4) and Figures (1-6).

Table 1: Clinical and demographic data of studied patients.

Variable		Number	Persentage
Age			
	Early childhood	5	25
	Late childhood	11	55
	Prepubertal	1	5
	Pubertal	3	15
Birth order			
	First	6	30
	Second	7	35
	Third	6	30
	Fourth	1	5
Consanguinity			
	Positive	3	15
	Negative	17	85
IQ			
	Mild	13	65
	Moderate	6	30
	Severe	1	5
Fragile X syndrome checklist Clinical score			
	≥19	3	15
	16- <19	3	15
	<16	14	70

Table 2: Clinical examination data for all index cases.

Item	Number	Percentage
Neuropsychiatric		
Speech problems	15	75
Hyperactivity	11	55
Poor eye contact	5	25
Autistic like behavior	3	15
Aggressive behavior	2	10
Epilepsy	3	15
Genital		
Undescended testicles	2	10
Skeletal		
Brachydactyly	1	5
Talipus equino varus	1	5
Kyphoscoliosis	1	5
Endocrinal		
Obesity	1	5
Dermatological		
Nail dystrophy	4	20
Abnormal pigmentation	2	10
Eye abnormalities		
Hypotelorism	1	5
Epicanthus	1	5
Upward slant	5	25
Synophrys	1	5
Ear abnormalities		
Large ear	11	55
Posteriorly rotated ear	3	15
Skull abnormalities		
Macrocephaly	6	30
Microcephaly	5	25
Elongated face	14	70
Prominent forehead	9	45
Nose abnormalities		
Depressed nasal bridge	2	10
Wide nostrils	1	5
Oral cavity		
High arched palate	13	65
Abnormal teeth	6	30

Table 3: Cytogenetic studies of our 20 mentally retarded cases.

Family serial number	Karyotype	Number of patients	Percentage of patients
1st			
Case 1	46,y,Fra(x)(q27-3)	1	5
Case 2	46,y,Fra(x)(q27-3)	1	5
2nd			
Case 1	46,fra(x)(q27-3),inv(y)	1	5
Case 2	46,fra(x)(q27-3),inv(y)	1	5
3rd - 11th	46 xy Normal karyotype	16	80

Table 4: Major clinical features in the four affected cases who expressed the fragile site on cytogenetic analysis.

Variable	Cases			
	1st	2nd	3rd	4th
Age group	Late childhood	Early childhood	Late childhood	Late childhood
Degree of mental retardation	Moderate	Moderate	Moderate	Mild
Dysmorphic features				
Macrocephaly	+ve	+ve	-ve	+ve
Prominent forehead	+ve	+ve	-ve	-ve
Long face	+ve	+ve	+ve	+ve
Large ears	+ve	+ve	-ve	-ve
High arched palate	+ve	+ve	+ve	+ve
Behavior				
Hyperactivity	+ve	+ve	+ve	-ve
Autistic	+ve	+ve	-ve	-ve
Poor eye contact	-ve	+ve	-ve	-ve
Speech problem	+ve	+ve	+ve	+ve

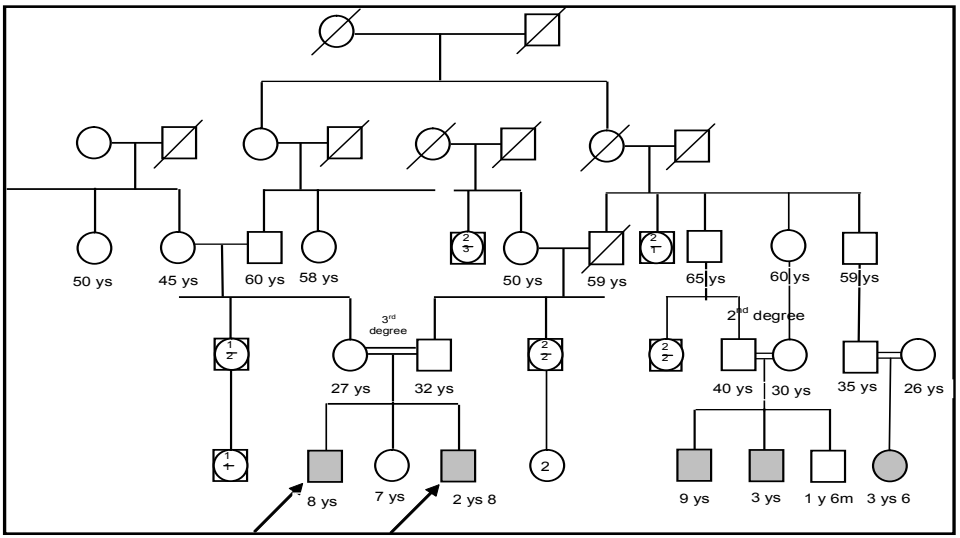


Fig. 1: The pedigree of a family with two similary affected cases , also, there are other affected relatives.

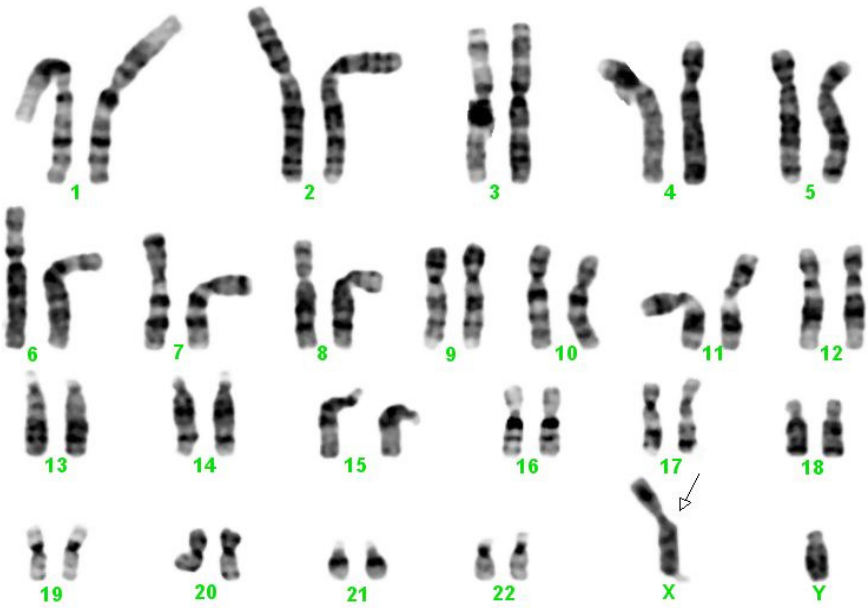


Fig. 2: A Karyotype of the 1st patient with X chromosome showing fragile site (it looks as non staining gap on terminal end of long arm of X chromosome.

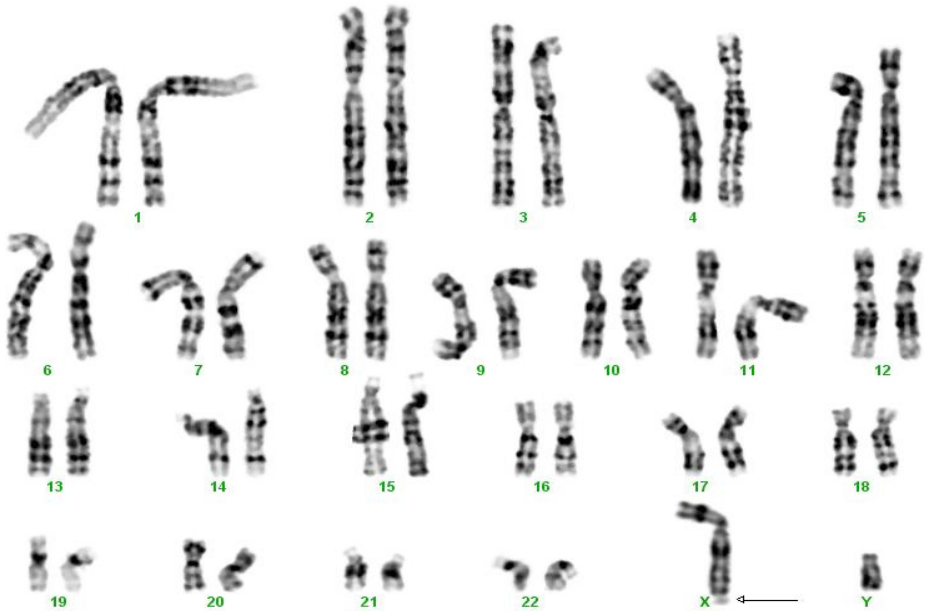
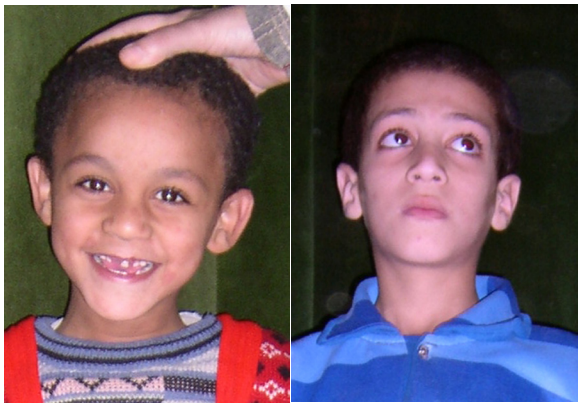


Fig. 3: A karyotype of the 2nd Patient with X chromosome showing fragile site (it looks as if the tip is breaking off but not quite separated).



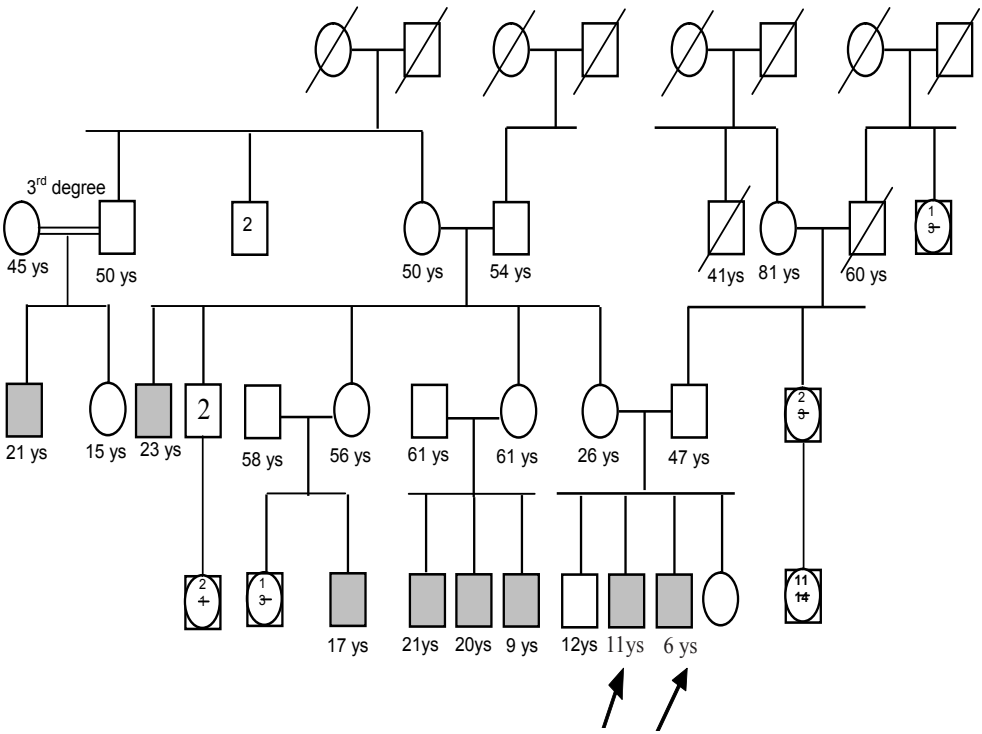


Fig. 4 : A family pedigree of the 3rd and 4th cases with similar affection.

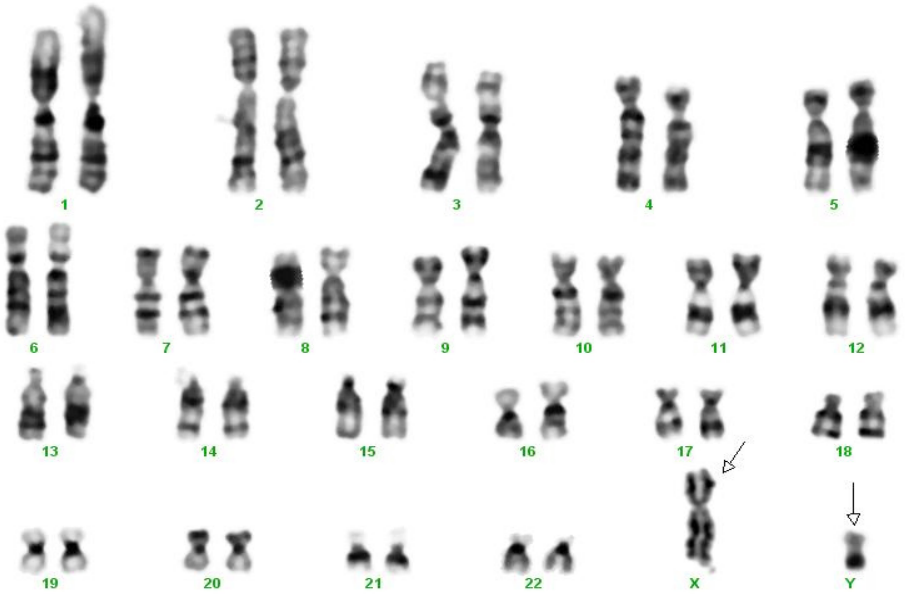


Fig. 5: A karyotype of the 3rd patient with X chromosome showing fragile site (It looks as non staining gap at the terminal end of long arm of X chromosome). also there is inversion of Y chromosome.

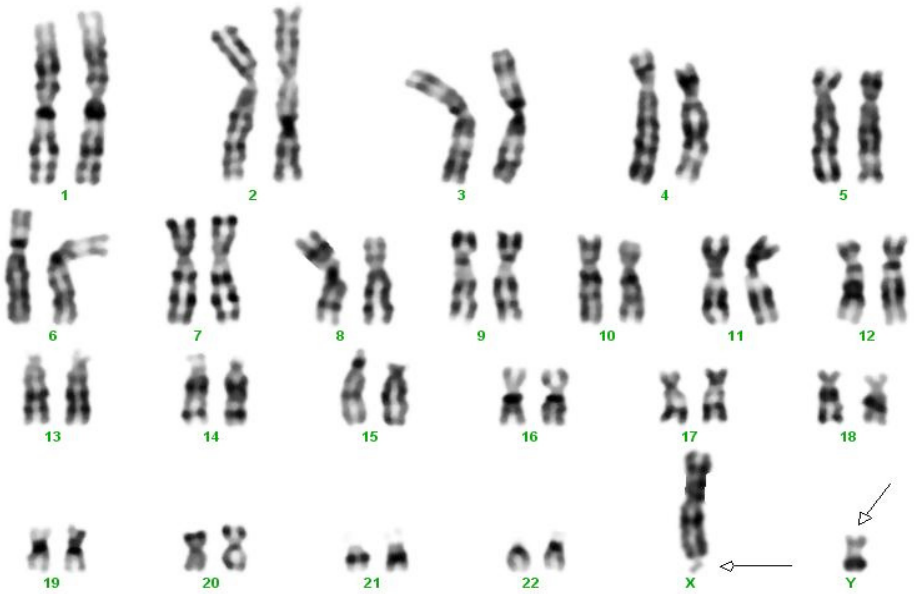


Fig. 6 : A karyotype of the 4th patient with X chromosome showing fragile site (It looks as non staining gap in at the terminal end of long arm of X chromosome). also there is inversion of Y chromosome.

DISCUSSION

Fragile X syndrome caused by a variability in tandemly repeated trinucleotide sequence CGG, near the 5` end of the FMR-1 gene with normal alleles having fewer than 45 repeats (5-44) while repeat sizes between 45 to 54 are considered in the grey zone. When the size of the repeat is between 55-200, it is considered a premutation allele. Two hundred repeats and above are considered in full mutation range.[11]

In this study, clinical information was recorded for all index cases. Their age ranged between 2 and 16 years (mean age 8.5 yr). The highest incidence (11 cases i.e. 55%) was in late childhood period.

The results were in accordance with Shevell et al.[12] who reported that the average age of diagnosis of fragile X-syndrome currently is eight years, reflecting subtle features in young children.

Positive consanguineous marriage was found in 15% of the cases. Nine families of the total eleven had positive family history; most of them were second degree relative males through maternal cousins.

This is in accordance with X-linked pattern of inheritance of the syndrome in which the phenotype is usually observed in the relatives of the maternal side of the family and males are more affected than females.[13]

As regards the degree of intellectual impairment of all index cases in our study; there was mild mental retardation in 13 cases (65%), moderate mental retardation in 6 cases (30%) and severe mental retardation in only one case (5%).

Among the four index cases who proved to be positive for fragile X syndrome by cytogenetic study, three cases were moderately mentally retarded while one case only was mildly retarded.

These data were in accordance with Lachiewicz et al.[14] who stated that mental retardation in fragile X-males varies from mild to profound with the most affected males being moderately retarded, the same results were reported by Hagerman et al.[15]

As regards the fragile X clinical checklist, it was found that three cases had scores >19 (15%), another 3 cases had scores 16-<19 (15%) while the remaining 14 cases had scores <16 (70%). These results were in accordance with Hagerman et al.[7], who reported that 45 percent of males with fragile X syndrome had a score of 16 or higher.

Regarding craniofacial and clinical data of our patients, it was found that elongated face (70%), high arched palate (65%), large or prominent ears (55%), prominent forehead (45%), macrocephaly (30%) and upward slanting palpebral fissures and microcephaly (25%) were the most common clinical findings, these results were in accordance with Bastaki et al.[16]

Macro-orchidism was not observed in our patients as most of our patients were prepubertal which was also reported by Fryns[17] and Hagerman[18] who stated that large testis is difficult to identify early in life.

Defective speech was observed in 15 cases (75%). The second most frequent behaviour disorder in our study was hyperactivity (55%), poor eye contact (25%), autistic like behavior (15%) while 10% of cases had aggressive behavior and epilepsy was found in 15% of cases.

Likewise, Bastaki et al.[16] found that 85% of fragile X cases had hyperactivity, 85% had defective speech and 45% has autistic like behavior.

In our study, cytogenetic studies revealed that normal karyotypes (46, XY) were present in 16 patients (80%), and four cases (20%) had positive fragile site on X-chromosome of whom 2 cases of one family had 46 y. Fra (X) (q27.3) and the other 2 cases also of one family had inversion of Y chromosome beside positive fragile site, 46, Fra (X) (q27.3), inv (y). The fragile site looked as non staining gap on terminal end of long arm of X chromosome (first, third and fourth patients) and as if the tip is breaking off but not quite separated in the second patient.

However, many authors reported a lower incidence of fragile X syndrome among mentally retarded patients.[16-19]

Our higher incidence may be due to the preselection of our patients based on the presence of clinical criteria of fragile X syndrome, which in turn increase the possibilities of finding fragile X positive cases.

Our four cases with positive fragile sites on X-chromosome were suffering from speech problems and poor eye contact (100% for each), most of them (75%) had moderate mental retardation and they were in late childhood period (75%), only one case was in early childhood, Alanay et al.[20] stated that although fragile X syndrome is generally regarded as the most common form of mental retardation, this underestimates its clinical extent since many individuals affected by the behavioral, emotional and/or learning disabilities of fragile X have IQs in the normal or border line range.[20]

Gabis L and Kesner Y added that using cognitive impairment as an inclusion criterion is a problem that is particularly marked for girls, with the majority having IQs within the normal range.[21]

On the other side, the clinical scoring checklist for these four patients was found to be more or equal to 19 in 2 cases of them (50%) and one case had score 16 to less than 19 and the last one had score less than 16.

These observations revealed the importance of application of clinical scoring for fragile X check list system in all mentally retarded cases as an important item for early diagnosis.

So, in any child with mental retardation or autism of unknown cause with considerable clinical scores for fragile X syndrome, the chromosomal study that identifies the fragile site at Xq27.3, in addition to other cytogenetic abnormalities could be useful because at least 5% of cytogenetic abnormalities has been seen in population with mental retardation examined for fragile X syndrome.[22,23]

Efforts should be concentrated on improving the diagnostic methods, fragile X scoring check list will increase the

diagnostic rate and subsequently the conventional support for the X linked mental retardation. Screening programs should be targeted at individuals who are at a higher risk. The various proposed strategies should include preconceptional testing and routine prenatal screening of all carrier pregnancies.

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