Fragile X syndrome clinical and associated comorbidities

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Background

Fragile X syndrome (FXS) is the most common inherited cause of intellectual developmental delay, affecting 1 in 4000 males and 1 in 8000 females. It is caused by expansion of CGG trinucleotide repeats in the 5' untranslated region of the fragile X mental retardation 1 (*FMR1*) gene that leads to hypermethylation. Fragile X represents with certain physical manifestations; however, a spectrum of subtle medical problems may occasionally be associated with these children.

Aim

The aim of this study is to highlight the prevalence of fragile X-associated subtle medical problems confirmed by bisulfate treatment and methylation-specific (MS)-PCR assay as a sensitive, accurate, and rapid technique.

Patients and methods

The current study included 32 Egyptian male children clinically diagnosed with fragile X symptoms. Their ages ranged from 4.2 to 11.9 years, with an average age of 7.5 years. All cases were subjected to thorough physical, clinical, and neurological examinations and confirmed by molecular analysis using conventional PCR, followed by bisulfite treatment and MS-PCR.

Results

Recurrent seizures were the main presenting complaint in 14 (43.75%) patients, autism spectrum disorder in seven (21.8%) patients, attention-deficit hyperactivity disorder in six (18.7%) patients, and delayed language development in four (12.5%) patients. Cardiac anomalies were found in five (15.6%) cases, four (12.5%) patients had strabismus, two (6.25%) patients had otitis media, and another two (6.25%) patients had gastrointestinal problems. Some patients exhibited more than one clinical presentation. MS-PCR of *FMR1* gene revealed full mutations with CGG repeats of more than 200 in 24 (75%) patients, and premutation alleles with CGG repeats ranged from 55 to 200 in eight (25%) cases.

Conclusion

Children with FXS should be subjected to specific and accurate checklist of physical, neurological, cardiac, ophthalmic, and ENT examinations. MS-PCR assay is a useful technique for rapid and accurate molecular diagnosis of FXS. This may allow early detection, diagnosis, and management of these subtle medical problems.

Keywords:

attention-deficit hyperactivity disorder, autism spectrum disorder, epilepsy, fragile X syndrome, full mutation, intellectual disability, methylation-specific PCR, full mutation

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Introduction

Fragile X syndrome (FXS) is the most common cause of inherited genetic intellectual developmental delay. It has been estimated that the prevalence of FXS is 1.5 per 10 000 of all cases of mental retardation, ~1: 4000 men and 1: 8000 women (Lozano *et al.*, 2016). Prevalence of FXS within the mentally subnormal males was found to be 6.4% in Egypt (Meguid *et al.*, 2007). It is reported that the average intelligence quotient of fragile X males with full mutation is 40% (Garber *et al.*, 2008). Clinically, children with FXS have certain physical features, which include prominent forehead owing to mid-facial hypoplasia with sunken eyes, large protruded cupped ears, strabismus, high-arched palate, and macroorchidism (Hagerman, 2008). There are other physical findings denoting connective tissue dysplasia including joint hyperextensibility, pectus excavatum, and flat feet and cardiac anomalies such as mitral valve prolapse (MVP) (Kidd *et al.*, 2014). It has been reported that a spectrum of subtle medical problems are commonly associated with children with FXS, for example, seizures, cardiac anomalies, strabismus, otitis media (OM), and gastrointestinal disorders (Baily *et al.*, 2008). Psychiatric disorders are also detected in children with FXS in the form of attention-deficit hyperactivity disorder (ADHD), autism spectrum disorder (ASD), delayed language development (DLD), aggressiveness, self-mutilation, and anxiety (Kaufmann *et al.*, 2017). As these subtle comorbidities may be

overlooked, pediatricians should be aware of them, so early diagnosis and proper treatment could be done. FXS is an X-linked dominant inheritance disorder, caused by mutation in CGG trinucleotide repeats in the 5' untranslated region (UTR) of exon 1 within fragile X mental retardation 1 (*FMR1*) gene located at Xq27.3 chromosome (Niu *et al.*, 2017). *FMR1* gene consists of 17 exons spanning 38 kb. There are four allelic forms depending on the expansion number of the CGG trinucleotide repeats: normal allele (5–45 CGG repeats), intermediate allele (also referred as gray zone or borderline) (45–55 CGG repeats), premutation allele (55–200 CGG repeats), and full mutation allele (>200 CGG repeats) (Ciaccio *et al.*, 2017). Other mutations of the *FMR1* gene such as deletions or point mutations have rarely been reported. The FXS mutations cause hypermethylation of the cytosine-phosphate-guanine island located in the promoter region of the *FMR1* gene, which results in transcriptional gene silencing and absence or remarkable reduction of FMR1 protein. This protein is important for proper neuronal morphology, cognitive development, and synaptic plasticity, and its absence leads to changing levels of ID (Santoro *et al.*, 2012). Males are more affected than females, as *FMR1* gene is located on the X chromosome (McLennan *et al.*, 2011). Different standard methods have been developed for diagnosis of FXS. Methylation-specific PCR (MS-PCR) assay is considered as a rapid, inexpensive, and effective technique for molecular diagnosis of patients assigned with FXS (Kanwal *et al.*, 2015). It is based on bisulphite treatment of DNA sequence, where unmethylated cytosine residues are converted to uracil, whereas methylated residues remain unconverted, followed by MS-PCR amplification of FMR1 promotor using primers specific for methylated in FXS cases (methylated) and unmethylated primer pairs in unaffected individuals (Chaudhary *et al.*, 2014). The aim of the current study is to screen subtle medical problems associated with FXS in Egypt. Bisulfate treatment and MS-PCR technique is a sensitive and accurate technique and of great help for precise diagnosis of FXS.

Patients and methods

Patients

The study was carried out according to the standards of the Egyptian government under protocols approved by the Medical Research Ethics Committee of the Egyptian National Research Centre, and informed consent was obtained from all patients' guardians. A total of 32 Egyptian male children clinically diagnosed with FXS were referred by Clinics of Medical Research of Excellence Centre, National Research Centre, Cairo, during the period from June 2015 till December 2017. Their ages ranged from 4.2 to 11.9 years, with an average age of 7.5 years. Positive consanguinity was detected in 18 (56.25%) cases.

Methods

Clinical examinations

Patients were subjected to thorough clinical and neurological examination and neuro-physiological tracing. All cases were subjected to full cardiac examinations, including echocardiography. Moreover, ocular, ear-nose and throat, and gastrointestinal examinations were done. Developmental assessment using Diagnostic and Statistical Manual of Mental Disorder (DSMMD) was also done for all patients. A detailed questionnaire was fulfilled by their caregivers to obtain a detailed early neonatal history.

Molecular analysis

Molecular analysis comprised two techniques: conventional PCR to amplify CGG repeats of the 5′ UTR and exon 1 of *FMR1* gene (Chong *et al.*, 1994) as well as Bisulfite treatment followed by Methylation-specific PCR (MS-PCR) (Chaudhary *et al.*, 2014).

Genomic DNA was extracted from 32 patients' peripheral blood using Thermo Scientific Gene JET Genomic DNA Purification Kit (Thermo Scientific, Waltham, MA, USA) according to the manufacturer's instructions. Concentration and purity of DNA was quantified using nano drop and stored in aliquots at −20°C. CGG repeats of the 5′ UTR and exon 1 of *FMR1* gene were amplified using Qiagen PCR-Core Kit (Qiagen, Dusseldorf, Germany and specific primers that were designed referring to genomic sequence (GenBank accession numbers≠NG_007529.1). The reaction was performed in a total volume of 25 µl and contained 100ng of genomic DNA, 200 µM from each of dATP, dCTP, dTTP, and 150 µM/50 µM dGTP/7'deaza-dGTP, 10 pmol fragile X forward and reverse primers, 1 × Q-solution, 1 × betaine as well as 2 U Qiagen Taq polymerase, and 1x buffer. The reaction was carried out using the following program: 99°C for 10 min (Hot start) followed by 35 cycles of 99°C for 1 min, 60°C for 90 s, 75°C for 2 min, and finally, extension at 75°C for 10 min. PCR amplicons were observed on 2% agarose, stained with ethidium bromide.

Bisulfite treatment of DNA was done using Qiagen EpiTect Bisulfite Kit (Qiagen) following the manufacturer's instructions. All MS-PCR reactions

were carried out in 25 µl volume, and each reaction contained 1 × buffer, 1 × Q-solution, 1 × betaine, 200 µM from each of dATP, dCTP, dTTP, together with 150 µM/50 µM dGTP/7'deaza-dGTP, 2 U of Qiagen polymerase, 500 ng of the bisulfite-treated DNA, and 10 pmol of each set of methylated and unmethylated primer pairs. Primers were designed according to Methprimer (Thermofisher scientific., USA) software. Thermal cycling conditions of methylated residues were as followed: denaturation at 95 ˚C for 5 minutes, 35 cycles at 95˚C for 1minute, 65˚C for 1 minute, 72˚C for 1 minute and extension at 72˚C for 10 minutes. Meanwhile, Cycles of unmethylated residues were denaturation of PCR reaction at 95˚C for 5 min, followed by 35 cycles of 95˚C for 1 minute, 58˚C for 1 minute, and 74˚C for 2 minute, then a final extension of 10 min at 74˚C and PCR fragments were analyzed by electrophoresis through 2% agarose gels stained with ethidium.

Results

We clinically suspected 54 cases had hyperactivity, psychomotor delay, intellectual disabilities, and mild variety of dysmorphic features including large cupped ears, high-arched palate, and sunken eyes. They also displayed some autistic-like features. Molecular analysis revealed that 20 of these cases showed trinucleotide expansion mutations and 12 showed premutations. The remaining 22 did not show any expansion in the trinucleotide repeats. Therefore, they were excluded from the study.

The 12 cases with premutation were clearly displaying milder features and mainly in the form of psychomotor delay and mild hyperactivity but lacked the dysmorphic features.

Clinical results

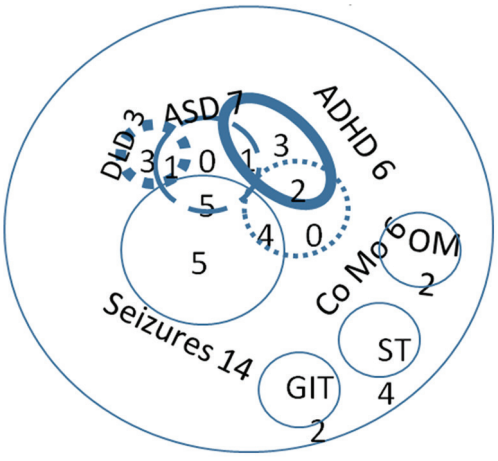
Recurrent seizures were the main presenting complaint in 14 (43.75%) patients. ASD was found in seven (21.87%) cases. ADHD and other psychiatric comorbidities were each detected in six (18.75%) cases, and DLD was present in four (12.5) cases. Other main presenting complaints were as follows: four (12.5%) patients presented with strabismus, two patients presented with recurrent OM, and recurrent abdominal disorders were detected in another two (6.25%) patients. Some patients exhibited more than one clinical presentation (Table 1 and Fig. 1).

Regarding the 14 patients with seizures, eight patients developed seizures at the age of 5–9 years with frequency of seizures ranging from 2 to 10 times/week in 10 patients. The main type of seizures among our cohort was complex-partial seizure with or without secondary generalization (Table 2 and Fig. 2). The frequency of psychiatric problems among the 14 patients who presented with seizures was ASD in five (35.7%) patients, ADHD in four (28.5%) patients, and DLD in two (14.3%) patients. Other psychiatric comorbidities, for example, aggressiveness, self-mutilation, and anxiety, were detected in only three (21.4%) patients (Table 3). Regarding MVP was detected in five (15.6%) patients among our cohort, as shown in Fig. 3. Two patients were confirmed to have aortic dilation. Proper neonatal history was recorded according to a detailed questionnaire of the parents. This revealed that 20 (62.5%) patients had low-birth weight, whereas only two patients were born prematurely. A total of 16 (50%) patients had abnormal ears, 10 (31.25%) patients had macrocephaly, whereas macroorchidism was detected in four (12.5%) patients (Table 4).

Molecular results

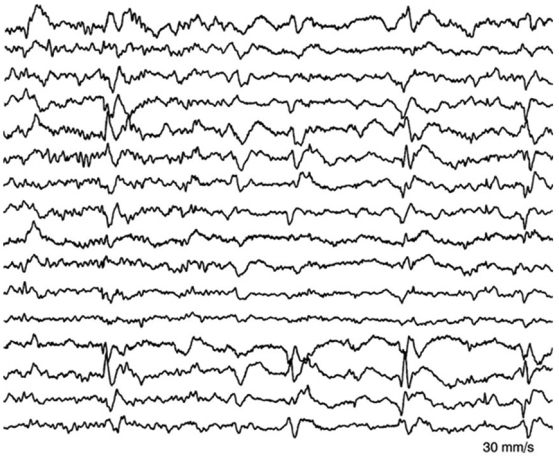
Molecular analysis revealed full mutations in the

Figure 1



Venn diagram presenting the main complaints among our cohort.

Figure 2



EEG changes of patient number 12 showing complex-partial seizures.

Table 1 The main presenting complaint and molecular diagnosis among our cohort

Case numbers	Age at onset	Consanguinity	Presenting complaint	Type of mutation
1	$11^{9/12}$	+	DLD/ASD	Full mutation
2	$9^{7/12}$	+	ADHD	Full mutation
3	$6^{6/12}$	-	Seizure/comorbidities	Full mutation
4	$4^{3/12}$	+	Seizures	Premutation
5	$10^{6/12}$	-	Strabismus	Full mutation
6	$4^{9/12}$	+	Seizures/ASD	Full mutation
7	$5^{8/12}$	-	ADHD	Full mutation
8	$8^{6/12}$	+	Seizures	Full mutation
9	$4^{2/12}$	+	Seizures/comorbidities	Premutation
10	$6^{3/12}$	-	Strabismus	Premutation
11	$11^{6/12}$	+	ADHD/ASD	Full mutation
12	$8^{8/12}$	-	Otitis media	Full mutation
13	$7^{3/12}$	+	DLD	Full mutation
14	$5^{5/12}$	+	Seizures/comorbidities	Full mutation
15	$6^{3/12}$	-	GIT	Premutation
16	$10^{6/12}$	-	Seizures/ASD	Full mutation
17	$11^{9/12}$	+	DLD	Full mutation
18	$9^{7/12}$	+	ADHD	Full mutation
19	$6^{6/12}$	-	Seizures/comorbidities	Full mutation
20	$4^{3/12}$	+	Seizures	Premutation
21	$10^{6/12}$	-	Strabismus	Full mutation
22	$4^{9/12}$	+	Seizures/ASD	Full mutation
23	$5^{8/12}$	-	ADHD/comorbidities	Full mutation
24	$8^{6/12}$	+	Seizures	Full mutation
25	$4^{2/12}$	+	Seizures/ASD	Premutation
26	$6^{3/12}$	-	Strabismus	Premutation
27	$11^{6/12}$	+	ADHD/comorbidities	Full mutation
28	$8^{8/12}$	-	Otitis media	Full mutation
29	$7^{3/12}$	+	DLD	Full mutation
30	$5^{5/12}$	+	Seizures/ASD	Full mutation
31	$6^{3/12}$	-	GIT	Premutation
32	$10^{6/12}$	-	Seizures	Full mutation

ADHD, attention-deficit hyperactivity disorder; ASD, autism spectrum disorder; DLD, delayed language development GIT, gastrointestinal.

Table 2 Illustrates the pattern of seizures amongst our 14 cases with epilepsy

	Full mutation 10 cases	Premutation 4 cases
Mean age at onset		
From 1 to 3 years	2	-
From 3 to 9 years	6	2
Above 9 years	2	2
Frequency of seizure		
<2/week	1	1
From 2 to 10/week	7	3
More than 10/week	2	-
Type of seizure		
Complex-partial	7	3
Generalized	2	1
Combined	1	-
Severity of seizure		
Mild	2	3
Moderate	7	1
Severe	1	-
Control of seizure		
Well-controlled	8	3
Poor-controlled	2	1

FMR1 gene of twenty four (75%) patients, whereas eight (25%) patients had permutations.

Conventional PCR of CGG repeats in the 5′ UTR and exon 1 of *FMR1* gene illustrated full mutation with more than 200 CGG repeats in twenty four patients and premutations with CGG repeats ranged from ~55–200 in eight patients, and normal individuals showed ranges of CGG repeats of 5–45 (Fig. 4). Meanwhile, MS-PCR amplification using two different sets of methylated primers demonstrated amplified products of 80 and 300-bp fragments in the 24 patients, indicating methylated alleles. However, MS-PCR amplification using two different sets of unmethylated primers showed amplified products of 80 and 300-bp fragments in normal males, indicating unmethylated alleles. In permutation carriers' males, both sets of unmethylated primers showed PCR fragment of 100 and 350 bp, with a range of 55–200 CGG repeats, respectively (Figs. 5 and 6).

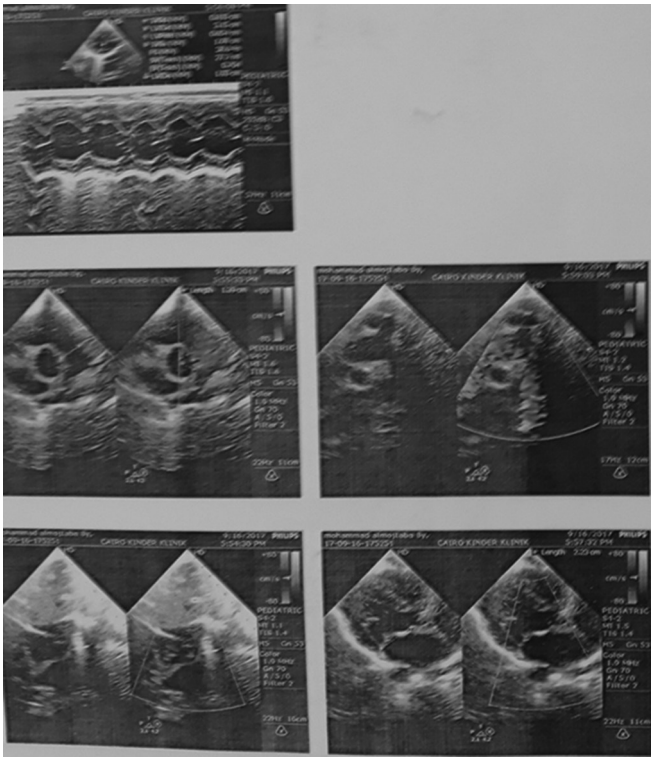
Discussion

Children with FXS are at risk of a wide range of subtle medical problems, and as they cannot communicate properly except by screaming and irritability, these problems

could be overlooked and improperly managed. Our study is designed to assess these subtle medical problems and analyze their prevalence to help for their early diagnosis, prevention, and management using MS-PCR assay. FXS (OMIM #300624) is an X-linked dominant disorder caused by expansion mutation in trinucleotide CGG repeats in 5′UTR and exon 1 of *FMR1* gene, leading to hypermethylation, which results in silencing of this gene. Males are more affected than females, which is clearly presented in our study (Dean *et al.,* 2016).

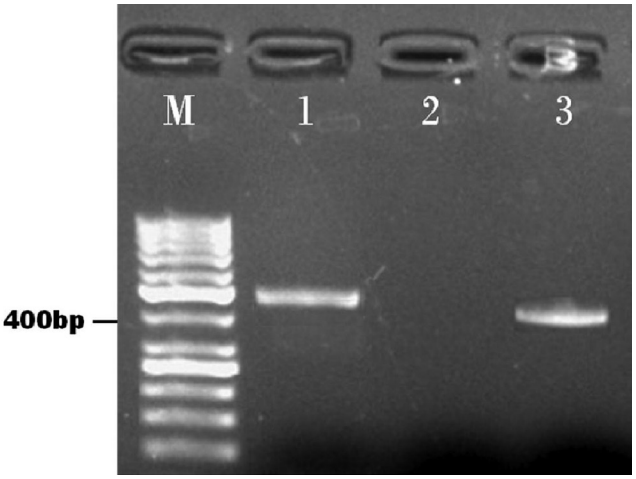
The expansion of the trinucleotide CGG with range 55–200 CGG repeats in *FMR1* gene results in premutation carriers and more than 200 CGG repeats results in full mutations (Salcedo-Arellanoa *et al.*, 2020). This is consistent with our molecular results. Fragile mental retardation protein (FMRP) is RNA-binding protein essential for dendritic translational receptor. Mutation in *FMR1* gene leads to subsequent lack of FMRP, which causes dysregulation of translation, resulting in excess immature dendritic spikes in children with FXS, resulting in synaptic

Figure 3



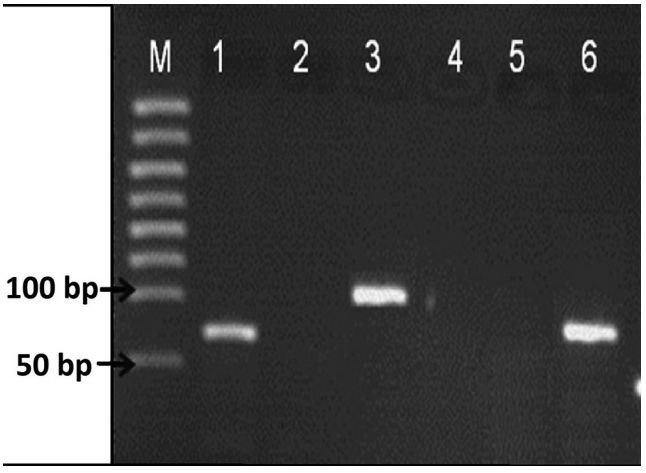
An echocardiographic image of case number 5 showing mitral valve prolapse.

Figure 4



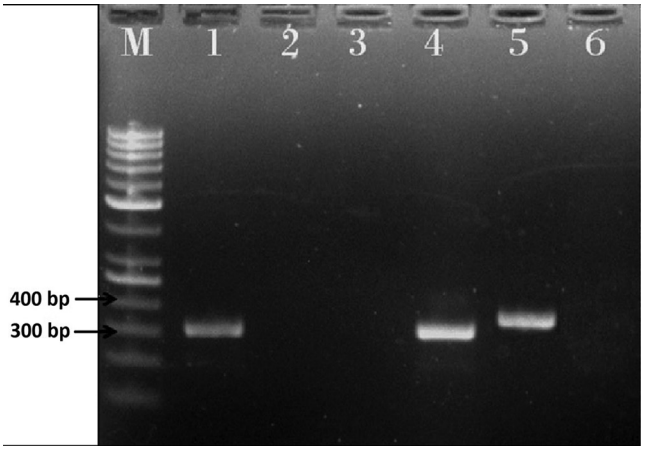
PCR results of CGG repeats (UTR) region and exon 1 of FMR1 gene. Lane M: DNA marker. Lanes 1: Premutation male carrier. Lanes 2: Patients with full mutation (fragments are always not visible on gels). Lane 3: Normal males.

Figure 5



MS-PCR using two sets of unmethylated and methylated primers. Lane M: DNA marker. Lanes 1, 3, and 5: unmethylated PCR product of normal male (PCR product of 80 bp), premutation male carrier (PCR product of 100 bp) and patient with full mutation, respectively. Lanes 2, 4, and 6: methylated PCR product of normal male, premutation male carrier and patient with full mutation (PCR product of 80 bp), respectively. MS-PCR, methylation-specific PCR.

Figure 6



MS-PCR using two sets of unmethylated and methylated primers. Lane M: DNA marker. Lanes 1, 3, and 5: unmethylated PCR product of normal male (PCR product of 300 bp), patient with full mutation and premutation male carrier (PCR product of 350 bp), respectively. Lanes 2, 4, and 6: methylated PCR product of normal male, patient with full mutation (PCR product of 300 bp) and premutation male carrier, respectively. MS-PCR, methylation-specific PCR.

Table 3 Frequency of psychiatric problems amongst our cohort

Complaints	Fragile X cases with seizures: 14 cases [*n* (%)]	Fragile cases without seizures: 18 cases [*n* (%)]
ADHD 6 cases	4 cases (28.5)	2 cases (11)
ASD 7 cases	5 cases (35.7)	2 cases (14)
Other comorbidities 7 cases	3 cases (21.4)	4 cases (22.2)
DLD 4 cases	2 cases (14.3)	2 cases (11)

ADHD, attention-deficit hyperactivity disorder; ASD, autism spectrum disorder; DLD, delayed language development.

Table 4 The medical problems amongst our cohort

Medical problems	*n* (%)
Neonatal history	
LBW	20 (62.5)
Prematurity	2 (6.25)
Physical examination	
LBW	20 (62.5)
Prematurity	2 (6.25)
Cardiac anomalies	
MVP	5 (15.63)
Aortic dilatation	2 (6.25)
Recurrent otitis media	2 (5.25)
Strabismus	4 (12.5)
Gastrointestinal problems	2 (6.25)

LBW, low-birth weight; MVP, mitral valve prolapse.

abnormalities and epileptic discharges. In the present study, molecular diagnosis using conventional PCR and MS-PCR revealed full mutations in the *FMR1* gene of 24 (75%) patients, whereas eight (25%) patients had premutations. These two molecular techniques were applied by Kanwal *et al.* (2015) who screened FXS in 400 Pakistan students, and only 13 individuals were normal to FXS. Omar *et al.* (2016) diagnosed FXS in 64 Egyptian males with fragile X symptom, using conventional PCR; this study revealed eight (12.5%) cases with full mutation and six (9.4%) cases have permutation. Moreover, PCR technique was used in the study by Meguid *et al.* (2007) when they screened FXS in 500 patients and showed the prevalence of FXS mutation among Egyptian males was 0.9 per 1000. MS-PCR was developed for diagnosis of methylation-related diseases including FXS. The advantages of this assay are in requiring a small amount of DNA, being inexpensive, and less time consuming. It is based on bisulphite treatment of DNA followed by MS-PCR to distinguish accurate full mutation alleles (Tassone., 2015). El-Ghanya *et al.* (2019), detected abnormal alleles in six (12%) patients of total 50 Egyptian males: three (6%) patients with full mutation and three (6%) patients with premutation carrier using MS-PCR. Chaudhary *et al.* (2014), carried out a study in Saudi Arabia on 53 males based on MS-PCR and showed that nine of them were affected by FXS. In Korea, Yim *et al.* (2008), reported four children of 64 children with full mutation showed significant developmental delay, cognitive dysfunction, and varying degrees of autistic behaviors, and only one boy was permutation carrier. Karunasagar *et al.* (2005) applied MS-PCR on 25 Indian patients and revealed one case with full mutation and one carrier state. This explains the high risk rate of epilepsy amongst children with FXS (Danesi *et al.*, 2018). This is in accordance with our results, which revealed that epilepsy is the main presenting complaint among our cohort. Of 32 Egyptian children with FXS, 14 (43.75%) presented in our study with epilepsy. The incidence of epilepsy in children with FXS varies in the literature, but generally epilepsy is one of the most common presenting complaint in children with FXS, and its incidence in FXS clinics or community hospitals ranges from 12 to 18% (Musumeci *et al.*, 1999; Berry-Kravis *et al.*, 2010; Lozano *et al.,* 2016). In referral neurology clinics, the incidence could be higher where epilepsy is usually one of the main reasons for referral, and this explains our high incidence of epilepsy in our cohort. The age of onset of epilepsy among our cohort ranged from 5 to 9 years, with a frequency of seizures between 2 and 10 times per week. Complex-partial seizures with or without secondary generalization was the predominant type of seizures and they were well controlled, which is in accordance with those reported by Berry-Kravis *et al.* (2010). On the contrary, Gauthey *et al.* (2010) reported that one-third of patients with FXS presented with generalized seizures and status epilepticus. However, patients with FXS may have abnormal EEG tracings in the form of abnormal centro-temporal spikes without any apparent seizures (Baily *et al.,* 2008). Among our cohort, seven (21.8%) cases showed ASD, which is lower than other reports. Hernandez *et al.* (2009) reported that FXS is associated with ASD in 30–54% of cases, which could be attributed to the standard research criteria used for the diagnosis of ASD among our cohort. The relationship between FXS and ASD may be explained by the fact that FMRP controls the translation of almost 30% of ASD genes (Hagerman *et al.*, 2017). The underlying molecular etiologies are intermingled, and targeted treatment of FXS may help ASD management (Loesch *et al.*, 2004). When FMRP levels are not significantly diminished, affected children may develop delayed language, but when FMRP level is severely diminished, the affected child may develop ADHD and/or ASD (Kaufmann *et al.*, 2017). Among our 14 epileptic cases, five (35.7%) of them developed ASD, which is in accordance with García-Nonell *et al.* (2008), and this

could be attributed to the presence of the same underlying pathology of synaptic dysfunction. This may be owing to the severely comprised neural conductivity as a common risk for both disorders, yet there is no evidence that epileptic discharges or EEG changes cause autism (Deonna and Roulet, 2006). There is also no evidence that treatment of seizures may change the clinical course of ASD (Spence and Schneider, 2009). Although the 14 patients with epilepsy were well controlled, yet the five cases with ASD did not show any improvement. Further research is needed to explain the relationship between seizures and ASD among patients with FXS. ADHD is a common presenting symptom among our patients, which is in accordance with Loranzo *et al.* (2016). Although all our patients were asymptomatic, yet full cardiac examinations, including M mode and two-dimensional echocardiography, were undertaken for all patients. Five (15.6%) out of them showed MVP. The incidence of MVP in FXS in the literature showed great variability ranging from 5.8% up to 55% (Crabbe *et al.*, 1993; Alanay *et al.*, 2007), whereas Fragile Clinical Research Centre (FXCRC) database using only clinical reports showed an incidence of only 0.8% (Kidd *et al.*, 2014). This variability could be attributed to the difference in the methods used to detect MVP, as some studies used only clinical examination, whereas others used full cardiac evaluation, including echocardiography. Moreover, the patients' age should be put in consideration as MVP in FXS is more common in adults. MVP is the result of loose connective tissue owing to abnormalities in the elastin fibers. Abnormal elastin fibers have been detected in cardiac valves and skin of with FXS (Hayek *et al.*, 2005). Although MVP has minimal complications, it may lead to infective endocarditis and rarely congestive heart failure. Dilatation of the aortic root is also found in patients with FXS, especially in adults. This aortic dilation proved neither to be progressive nor to have aneurismal formation (Kidd *et al.,* 2014). Although the MVP in FXS is asymptomatic in children, yet we recommend subjecting all patients with FXS to careful cardiac examination, including echocardiography for early detection of any cardiac anomalies. Strabismus is one of the main ocular complaints among our cohort. Of the 32 patients with FXS, four (12.5%) presented with strabismus, which is similar to the report by Sarvananthan *et al.* (2009). The incidence of strabismus may be higher owing to selection bias and improper ophthalmic examination for these irritable children. Therefore, meticulous and specialized pediatric ophthalmologic examination should be done for all children with FXS to detect and manage any ocular abnormalities. Recurrent OM was the main presenting complaint in only two (6.25%) patients among our cohort. This is lower than the result reported by Lieberthal *et al.* (2013), who reported that OM occurs at a higher rate among children with FXS. The cause of recurrent OM among children with FXS may be related to the cranio-facial abnormalities with long face, which may affect the angle of the Eustachian tube. These anatomical changes lead to collapsible Eustachian tubes with fluid stagnation, which cannot be well drained. This impairment and stagnation of fluid in the Eustachian tubes will cause bacterial overgrowth and infection (Hoffman *et al.*, 2013). Any sign of redness, decreased motility of tympanic membrane, fever, vomiting, and/or headache should direct the attention of the pediatricians to OM. Early examination is essential for children with FXS with irritability, screaming, or any sleeping disorder. Recurrent OM may lead to more serious complications, including decreased hearing, DLD, and behavioral problems. Proper meticulous ear examination should be done, and prompt treatment should be started with antibiotic administration. If there is no response, early tympanostomy and tube replacement may be needed. Vaccination against pneumococci and influenza virus is recommended for all children with FXS with recurrent OM. As children with FXS are mostly nonverbal and cannot communicate properly, pediatricians should carefully search for any underlying medical problems that cause irritability or any behavioral changes. Regarding our cohort, only two (6.25%) patients presented with gastrointestinal troubles in the form of abdominal pain, constipation, and/or diarrhea and failure to thrive. Our result is lower than that reported by Pang and Croaker (2011). These gastrointestinal problems may be also owing to connective tissue disorders. According to the meticulous detailed questionnaire of the parents, 20 (62.5%) out of 32 patients with FXS had low birth weight. This is in contrast with other reports by Partington (1984), who reported that the mean birth weight of infants with FXS is higher than their siblings. Our results may be attributed to the poor maternal health and the low socioeconomic status. Large head circumference was detected in 16 (50%) cases. This result is in accordance with other reports which proved that head circumference of infants with FXS tends to be higher than the 50th percentile (Lachieweiz *et al.*, 2000). Macroorchidism was detected in only 10 (31.25%) cases, which is lower than other reports (Turner *et al.*, 1980), which could be attributed to the young age of our cohort, as they all were prepubertal.

Recommendations

It has been reported that a spectrum of subtle medical problems are commonly experienced by children having FXS. Children suffering from Fragile X syndrome should be subjected to full physical and medical examination,

in addition to, neurological, neuro-physiological tracings, behavioral assessment, detailed cardiac examination including echocardiography screening and molecular analysis using MS-PCR as an accurate and rapid technique for diagnosis of fragile X syndrome. Also, detailed ophthalmic, ENT, and gastrointestinal examinations should be undertaken. It seems important for certain tests e.g. cardiac examinations to be followed up routinely to detect any emerging medical problem.

Therefore, this study will provide the pediatricians with updated data and prevalence of these subtle medical problems to allow early diagnosis, proper management, and subsequently improve the quality of life of these children and their families.

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Nil.

Conflicts of interest
There are no conflicts of interest.

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